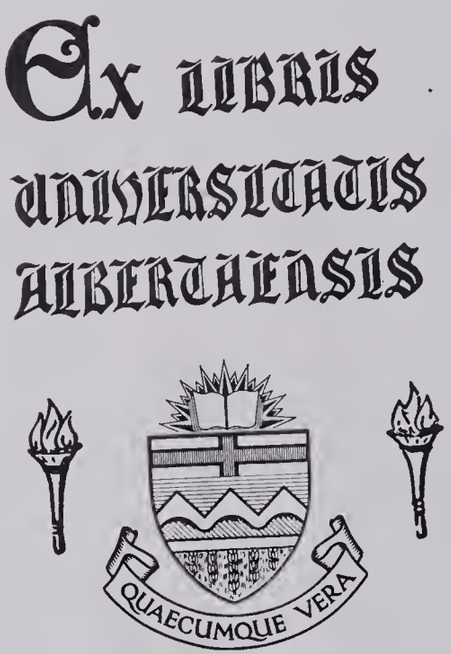

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CONSTITUENTS OF *ASARUM CANADENSE*

by

© JAMES ALLAN OGILVIE B. Sc.

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

FACULTY OF PHARMACY

EDMONTON, ALBERTA

SPRING, 1969

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Constituents of Asarum Canadense" submitted by James Allan Ogilvie in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

In a survey of the constituents of the volatile oil of Asarum canadense, it was found that doubt existed as to the quantity and nature of the components actually present.

The present investigation of the volatile oil of A. canadense resulted in the isolation by preparative gas chromatography, of 25 components out of a total of 30 separated by analytical gas chromatography. Ten components were positively identified, and five were tentatively identified.

The following constituents were positively identified: myrcene, β-pinene, linalool, linalyl acetate, α-terpineol, bornyl acetate, eugenol, methyleugenol, cis-methylisoeugenol, trans-methylisoeugenol. The following constituents were tentatively identified: β-ocimene, 2,3,4,5-tetramethoxyallylbenzene, cis-2,3,4,5-tetramethoxypropenylbenzene, trans-2,3,4,5-tetramethoxypropenylbenzene, aristolone.

This is the first time that β-pinene, cis-methylisoeugenol, and trans-methylisoeugenol have been reported for this oil.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the valuable advice and guidance offered by Dr. R. A. Locock and Dr. R. T. Coutts during the course of this investigation. He also wishes to thank his colleagues in the faculty for technical assistance and interest in the project.

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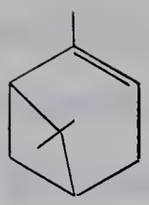
1. INTRODUCTION

The purpose of this thesis is to report the result of a systematic investigation carried out on extractives of Asarum canadense.

Aristolochiaceae consists of about six genera and 200 species. The genus Aristolochia accounts for about 180 of these species (1). The Asarum genus includes such species as: A. arifolium, A. blumei, A. canadense, A. caudatum, A. europeum, and A. sieboldi. Although most investigations have been carried out on A. europeum (2-7), some work has been done on the others; mainly on their essential oils.

A. caudatum is a perennial plant, possessing stem-like rhizomes and having a penetrating, ginger-like odor, and a spicy, acrid taste. Burlage (8) reported the presence of five constituents in the essential oil, and identified three of them as pinene, methyl-eugenol and asarone. Pinene (I) was identified by conversion to its nitrosochloride and subsequent conversion of the latter to the nitrol-piperidide. Methyleugenol (II) was identified by means of its bromination product, tribromomethyleugenol and by oxidation of the latter to veratic acid. The third compound identified was asarone (III). The only evidence given for its identity was its boiling and melting points, and the fact that its presence was expected because it was found in other Asarum species. The two constituents not completely identified were a phenol, which was thought to be eugenol (IV), and a blue oil (bp 230° / 20-40 mm) which had all the properties of azulene.



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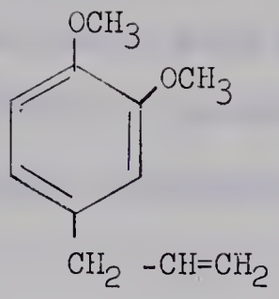

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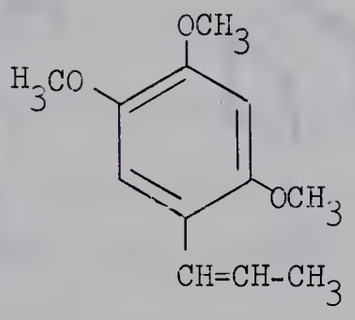

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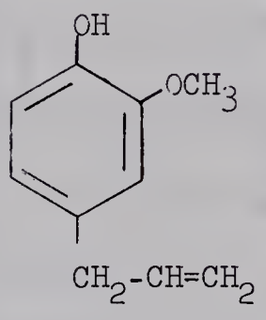
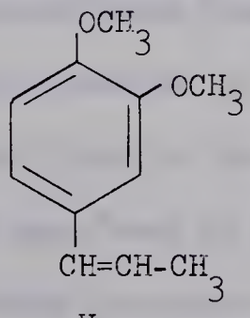


IV

The article by Burlage (8) referred to previous work done on the constituents of A. arifolium (9) and A. blumei (10). A. arifolium was was reported to contain pinene, eugenol, methyleugenol, methylisoeugenol (V), asarone, a sesquiterpene, an unidentified phenol, and safrole (VI). The only reported constituents of A. blumei were eugenol, safrole and a terpene.



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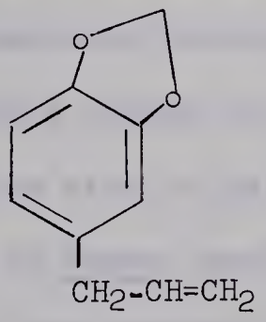
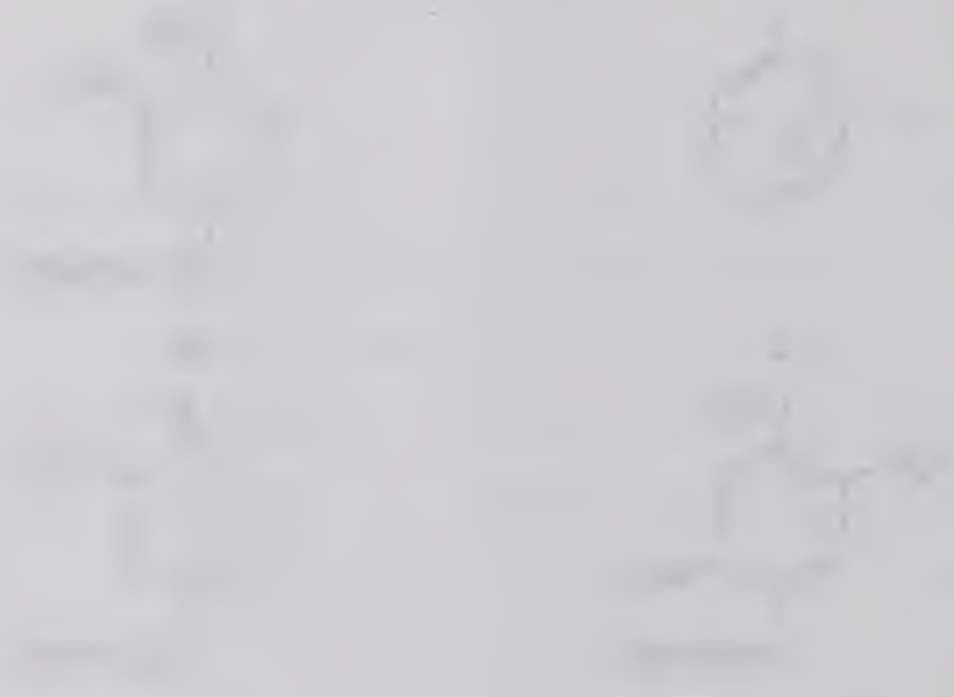
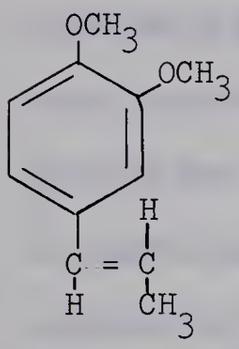


VI

A. europeum (hazlewort, wild nard, asarabacca) is an acrid, herbaceous perennial plant which grows in woods and shady locales in Europe, Siberia and the Caucasus (11). Stahl and Jork (2) have



reported the most important constituents of A. europeum as: trans-methylisoeugenol (VII); asaron; bornyl acetate; and a group of terpenes. The terpenes reported present were α-pinene (VIII), β-pinene (IX), myrcene (X), limonene (XI), camphene (XII), phellandrene (XIII), and terpinene (XIV).



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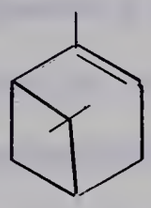

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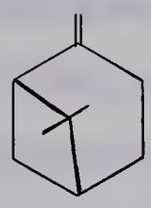

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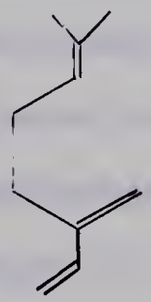

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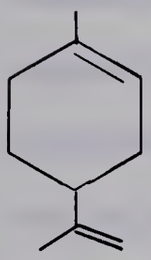

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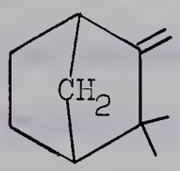

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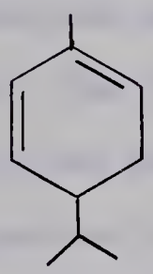

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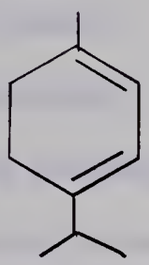
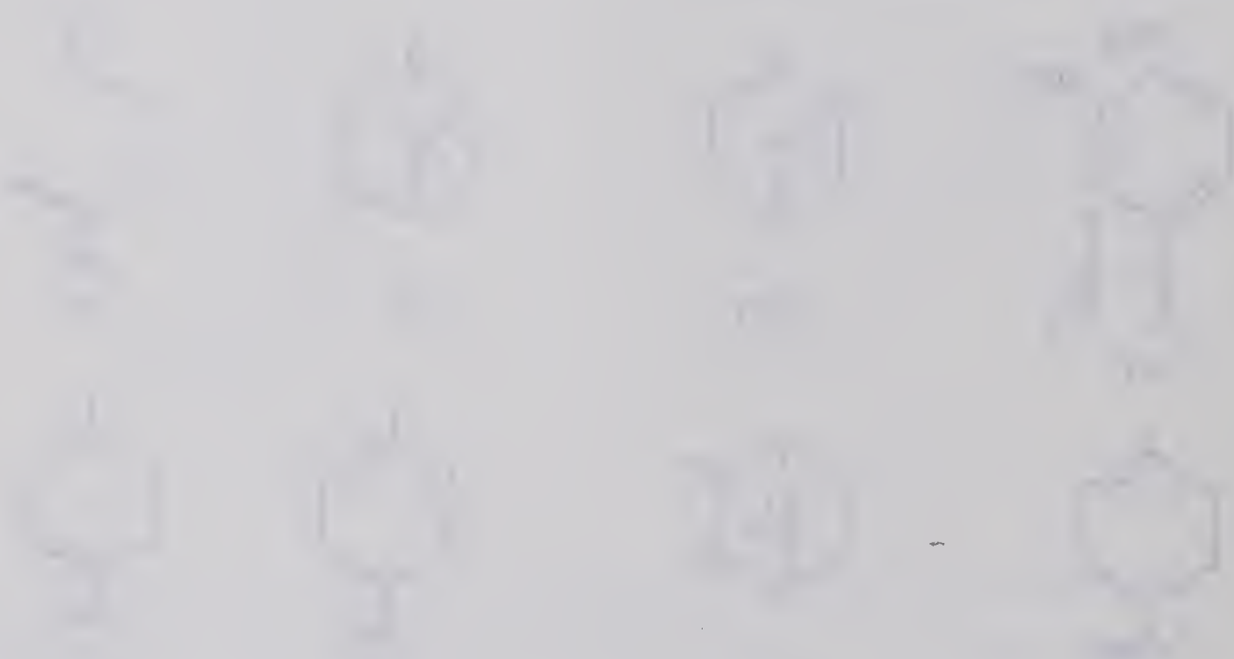
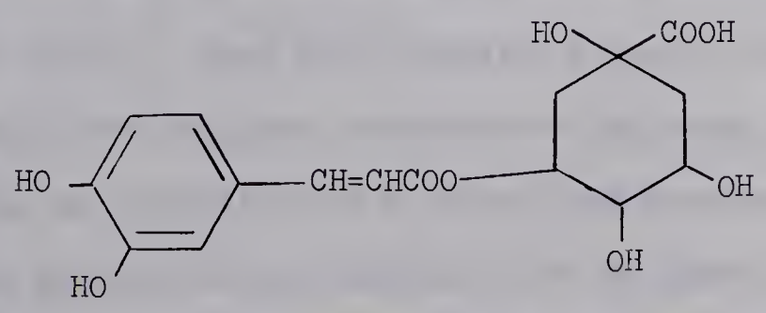


XIV

An earlier report by Gero (12) in 1928 reported the presence in A. europeum of "asaraldehyde" which possessed the formula $C_{10}H_{12}O_4$ and had a melting point of 114°; its oxime melted at 138°. He also mentioned diasarone as being present in the essential oil. No further mention of these compounds being present in A. europeum can be found in the literature. At the time of this writing there have been no alkaloids reported in Asarum species. Gracza (3), using the method of Webb (13), and the screening test of Prudhomme (14), could not detect the presence of alkaloids in different samples of A. europeum. In further work on A. europeum, Gracza (4) discovered the presence of 15 amino acids in the leaves and roots. The total amino acid content was 0.43%. The amino acids



present were lysine, serine, arginine, aspartic acid, asparagine, glycine, glutamine, glutamic acid, proline, tyrosine, tryptophan, phenylalanine, methionine, valine, and histadine. Gracza (4,5) has also reported the presence of carbohydrates in A. europeum. In the post-flowering period, the sugar content of the rhizomes equalled 3.87% and leaves 1.17%. The starch and cellulose content were found in rhizomes to be 1.65% and 8.52%, and in the leaves to be 0.3% and 6.05% respectively. By using thin layer chromatography he identified glucose, fructose, and sucrose. In 1965 Gracza (6) isolated a hydroxy-carboxylic acid from A. europeum. After extraction of the plant he used two-dimensional paper chromatography to separate the extraction mixture. The first dimension was developed with Partridge mixture (15), and the second dimension with aqueous acetic acid. The chromatogram was dried under ultraviolet irradiation in ammonia vapor. With this method flavanoids developed brown spots, tannins yellow spots, and an oxy-acid developed a blue spot. This acid he considered to be chlorogenic acid (XV). In 1964, Gracza (7) reported the isolation of β-sitosterol from the lipid fraction of the leaves of A. europeum.



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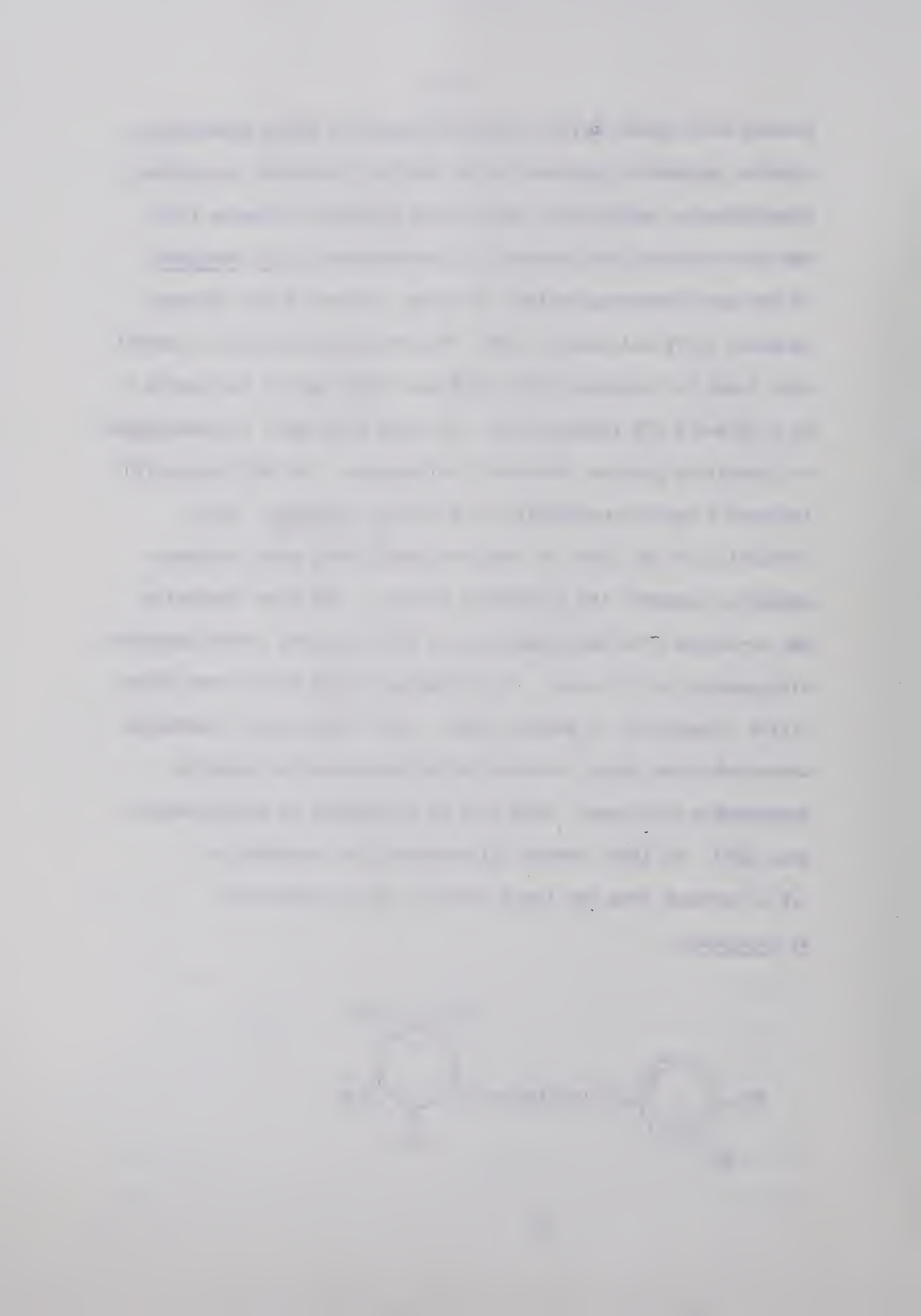
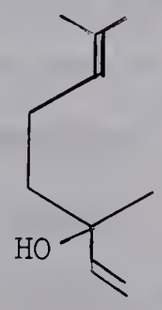


A. canadense (Canadian snake root, wild ginger) is a low perennial herb, possessing an aromatic, bitter rhizome and root system. The plant is native to the rich woodlands of North America. Commercial samples are obtained mainly from Virginia, North Carolina, Indiana, and Michigan (16). The essential oil of A. canadense has been investigated by two groups. In 1880, Power (17) published the results of his investigation into the volatile oil of Canadian snake-root. He reported that he had isolated a terpene, $C_{10}H_{16}$ bp 163-166°, and two fragrant alcohols of the same empirical formula, $C_{10}H_{18}O$. One of the alcohols (bp 196-199°) possessed an odor similar to coriander, and he named it asarol. The other alcohol (bp 254-257°) had little odor and analysed after oxidation as $C_9H_{10}O_4$. This oxidation product was subsequently (18) shown to be veratic acid. Veratic acid was the known oxidation product of methyleugenol. Power (17) collected a fraction which distilled between 275-350°. This fraction contained a deep blue oil which was not further characterized. Power also collected a large amount of acetic acid, which he concluded was combined with the two alcohols in the form of acetic esters. Power continued his investigation of Canadian snake-root oil, and in 1902, together with Lees, published (18) a report which further elucidated the components of the oil. They first isolated a nearly colorless, oily liquid, which when analysed, indicated an empirical formula of $C_9H_{12}O_2$. This was classified as a phenol, and because a clove-like odor was detected during liberation of the phenol from its alkaline solution, it was suspected that the phenol contained some eugenol.

After tests with ferric chloride it was concluded that the presence of eugenol in the phenol fraction was highly improbable, as the color developed with the phenol and ferric chloride was violet. Eugenol, under the same conditions, would have given a green color. When distilling the above-mentioned phenol, a solid substance separated in the condenser. The solid had a melting point of 60-61°, and was identified as palmitic acid. Pinene was the next component which Power identified. He concluded that the pinene isolated was a racemic mixture of the (+) and (-) forms due to its low optical rotation. It was identified by the formation of its nitrosochloride and by the conversion of the latter to the nitrolpiperidide. Power suspected that limonene might be present in Canadian snake-root oil, and a search was made for it. A bromine determination indicated only a dibromide, and not a tetrabromide which would form with limonene. Thus pinene was the only terpene which could be detected in the oil. Power isolated and identified linalool (XVI) which corresponded to the alcohol he named asarol in his earlier report. The next fraction identified boiled between 203-208° . By gentle oxidation and vacuum distillation a crystalline substance was obtained which melted sharply at 175°. This compound Power considered to be borneol (XVII). Its identity was confirmed by the preparation of the oxime after oxidation to camphor.



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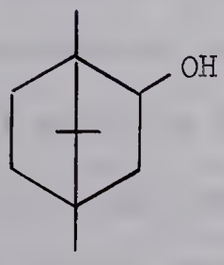
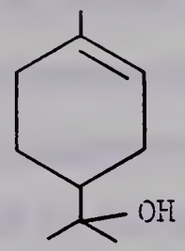


XVII

The next component identified by Power was terpineol (XVIII). Its identity was confirmed by its conversion into dipentene dihydriodide by hydriodic acid. A mixed melting point with dipentene dihydriodide prepared from pure crystalline terpineol was undepressed. Geraniol (XIX) was isolated from the fraction of Canadian snake-root oil which boiled between 222-235°. Its identity was confirmed by the formation of a diphenylurethane derivative, formed by treating the oil with diphenylcarbamyl chloride in the presence of pyridine. The major fraction of the oil boiled between 245-260°. On further distillation the fraction which boiled between 250-256° was collected as a colorless and nearly odorless liquid. When analysed the empirical formula $C_{11}H_{14}O_2$ was indicated. By reacting this liquid with bromine, the crystalline bromoeugenol methyl ether dibromide was obtained, which confirmed the presence of methyleugenol in the oil. During the investigation of the snake-root oil Power searched for the presence of methylisoeugenol, but its presence was not detected. Power could not identify the fraction of Canadian snake-root oil that boiled between 212-217° (60 mm), but after a molecular weight determination (freezing-point depression of phenol) gave a value of 214, he felt that it could be a sesquiterpene alcohol with the formula $C_{15}H_{26}O$ (mol. wt. = 222). Because the elemental analysis found for this liquid did not agree with that required for a sesquiterpene alcohol, he came to the conclusion that the fraction contained more than one substance. Power isolated a small amount of a dark colored, highly aromatic oil. He assigned the provisional empirical formula $C_{14}H_{20}O_2$, and felt that it was a lactone. The only other fraction

of Canadian snake-root oil found by Power during this investigation, was a mixture of fatty acids intermediate between acetic and palmitic acids.



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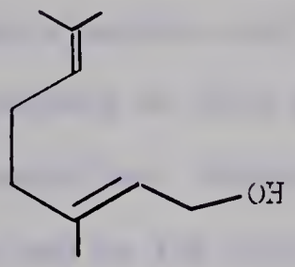
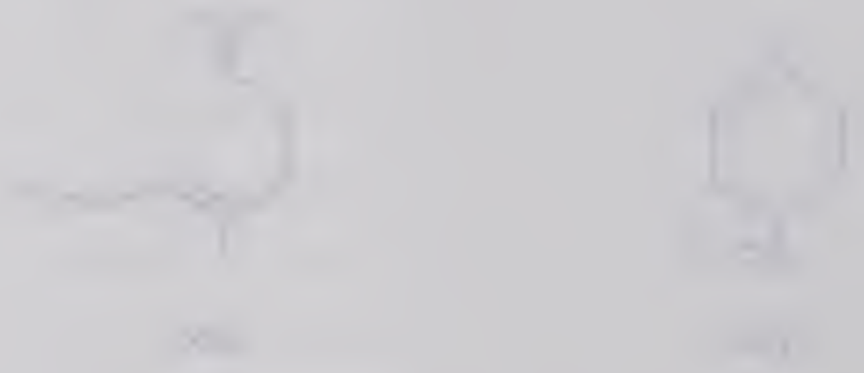
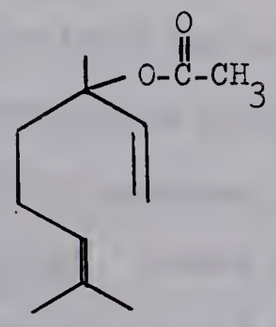


XIX

There was no further work reported on the constituents of A. canadense until 1967 when Bauer (19) published the results of his research into the volatile and non-volatile oil of the heptane-soluble fraction of the rhizomes. Using gas chromatography he determined that there were 13 components in the steam-volatile oil. Two compounds, present in trace amounts, were unidentified, and the remaining 11 were identified by various physical and chemical means. Limonene was identified by its mass spectrum which was identical to the published spectrum (20). Attempts were made to isolate pinene, because of the report by Power (18), but no evidence of its presence could be found. Three alcohols were isolated and identified (19). Geraniol was the most abundant (7.2%) in the oil, and was identified by its mass spectrum. α-Terpineol was isolated in 0.4% yield from the oil, and was identified by its mass and NMR spectra. The third alcohol isolated by Bauer was linalool. It was identified by its mass spectrum which contained the previously noted (21) unexplained peak at m/e 155 $(M+1)^+$. The identity of linalool was confirmed by its NMR spectrum (19). The major ester isolated by Bauer was linalyl



acetate (XX). The mass spectrum of linalyl acetate was typical of terpene esters, many of which produce a mass spectrum that does not exhibit a molecular ion peak (22). The first peak that appeared was the $(M-60)^+$ ion at m/e 136, which indicated the loss of acetic acid from the molecular ion (19). Bauer made a careful search for aromatic compounds because of their reported occurence in other species. Four aromatic compounds were isolated and identified. Methyleugenol was identified by its mass and NMR spectra, and by its oxidation to veratic acid. Methyleugenol was the major component of the steam-volatile oil. A small amount of elemicin (XXI)was isolated and was identified by matching its spectra with those of an authentic sample, and also by its oxidation to 3,4,5-trimethoxybenzoic acid. The third aromatic compound isolated by Bauer was 2,3,4,5-tetramethoxyallylbenzene. The only other aromatic compound which was isolated was the aldehyde, 3,4,-dimethoxycinnamaldehyde, which was identified by its mass and NMR spectra. Bauer isolated the compound aristolone (XXII) from the steam-volatile oil and the non-volatile residue. It was identified also by its mass and NMR spectra, and also by the formation of its 2,4-dinitro-phenylhydrazone. The last compound identified by Bauer was isolated from the steam non-volatile residue and was identical to an authentic sample of β-sitosterol.



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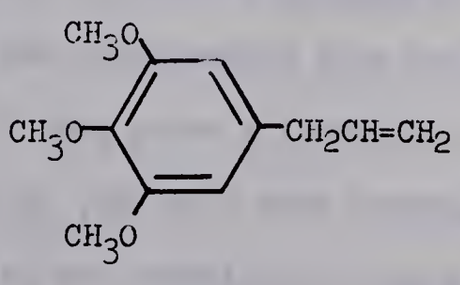

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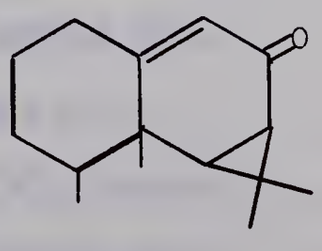
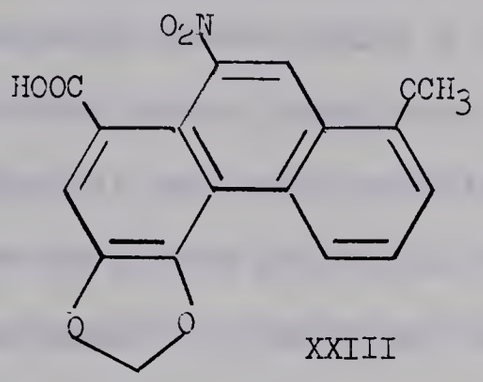


XXII

A. sieboldi is a member of the Asarum genus, which from the literature (23-27) appears to be found mainly in the Far East. A report in 1935 (25) stated that the essential oil of this plant contained a small amount of organic acid, which could be the palmitic acid that was reported present in 1930 by Takahashi (27). Methyleugenol appeared to be the major component of the oil, as one report stated the quantity as 50% (27). The presence of a phenol was reported in 1930, but only the empirical formula ($C_{10}H_{10}O_4$) was given (27). A report in 1935 (25) also listed a phenol. No formula was given, but its melting point was reported as 110° and that it formed an acetate which melted at 129-130°, and a benzoate which melted at 124°. Pinene was the only terpene present in A. sieboldi (27). The only other compound isolated from this plant was a ketone. In one report it was referred to as an "asaryl ketone" (27); a later report (25) did not name the ketone, but stated that it formed a semicarbazone melting at 182°.

In a general search for antibiotic substances involving the testing of 2,300 green plants, extracts of A. canadense and A. europeum demonstrated weak activity against Staph. aureus but not against E. coli (28). In 1946 Cavallito and Bailey (29) described the isolation of two antibacterial substances from A. canadense. Substance "A" was the more potent of the two and was assigned the tentative formula $C_{21}H_{20}O_8N_2S$. Substance "B", a lemon-yellow acid, was considerably less active against Gram positive organisms. It was given a tentative formula of $C_{16}H_{11}O_7N$. Coutts et al (30) felt that substance "B", isolated by Cavallito and Bailey was identical to the aristolochic acid

(XXIII) which they had isolated from various Aristolochia species. Only recently aristolochic acid has been isolated and identified from A. canadense (31).



XXIII

A brief mention must now be made of the medicinal uses of Asarum species. The use of these plants as a drug goes back as far as at least the middle of the nineteenth century (32). References to their use in China and Japan (23-26) would indicate they were used prior to then. The Chinese drugs Dai-Sin and Si-Sin were obtained from A. sieboldi (23). So, also, was the drug called Lsi-Lsin (24). The similarity of the last two mentioned drugs suggests that the references are to the same preparation. Preparations from the rhizomes of A. sieboldi have also been used in Japan under the title of To-Sai-Shin, as expectorants and diuretics (25,26). A. canadense has been used medicinally in eastern Canada and the north eastern United States. At one time it was largely used in perfumery (33), but it has also been used as an aromatic stimulent tonic (32), which was said to have diaphoretic properties. One of its earliest uses was as a masticatory, while the rhizomes were sometimes chewed to sweeten the breath (34). A. europeum, by its uses in Europe, appears to have been a much stronger drug. The root and leaves were reported to be powerfully emetic and cathartic, but were used almost exclusively

as an errhine (35,36), and as a sialogogue (36). Asarone, a constituent of the volatile oil of A. europeum was tested in 1930 on laboratory animals (37). Experiments demonstrated that asarone, when administered internally, either orally or subcutaneously, exercised a narcotic effect without being toxic. Experiments with the whole oil showed that it was toxic, producing hyperemia in all organs, inflamation of the kidneys and uterus, and finally death. These results probably explain to some extent the use of A. europeum as an abortifacient. Its use as such has been reported in Romania (26). Recently allantoin has been isolated and identified from A. europeum. The therapeutic effect of the plant on stomach and duodenum ulcers has been reported due to the activity of allantoin (38).

Although the investigations by Power (18) and Bauer (19) into the composition of the volatile oil of A. canadense had appeared to characterize the oil sufficiently, preliminary gas chromatograph experiments performed prior to Bauer's recent report (19) indicated that the oil contained more than the 13 components. For this reason, and because of the contradictions between the results of Power and Bauer, it was felt that further investigation of this plant was warranted.

II. DISCUSSION

Canadian snake-root oil is available commercially. For the present study, two samples of the oil were purchased. One was obtained from Dominion Herb Co. and will be referred to as oil A. The other sample was obtained from British Drug Houses and will be referred to as oil B. A comparison of the commercially available oils was to be made with freshly distilled oil, and for this purpose a quantity of dried, milled rhizomes of A. canadense were steam distilled to obtain a light yellow fragrant oil in 1.24% (w/w) yield, which will be referred to as oil C. An alternate method was tried to determine which method would give the best percentage recovery of volatile oil. A pentane extract of the rhizomes was steam distilled and yielded only 0.166% (w/w) of oil C.

Sample A of the oil was analysed by gas liquid chromatography. Bauer reported (19) using two columns for preparative GC which resolved the oil in 13 components. The two columns were a 20% SE 30 column, and a 20% DEGS column. For the present investigation a 10% SE 30 was available and this was the first column tried. It resolved the oil into 19 components (Table 4). Four other columns were tried (Polyphenol ether; Carbowax 20 M; LAC-728; and Diisodecyl Phthalate) but the resolution of the oil in each case was poor.

To compare the three samples of the oil (A,B, and C) each was chromatographed (GLC) under identical conditions using the SE 30 column. Each sample of the oil appeared to contain the same 19 components according to retention times, but the quantities of each varied widely between the samples. (A more detailed discussion of the comparitive results of the three samples will follow later in this report). As oil C appeared to contain greater than 80% of the 12th component eluted from the column, and because the oils were qualitatively similar, and oil A appeared to have the most even distribution, it was decided to use oil A for the determination of the components of oil of Canadian snake-root.

Knowing the number of components in the oil and their retention times, an attempt was made to separate the oil into an arbitrary number of fractions each containing only a few components. A condenser packed with glass beads was employed as a fractionating column. The oil (A) was distilled under reduced pressure and five fractions were collected. Each of these fractions was chromatographed (GLC) on the SE 30 column and the results (Table 3) showed

that separation was poor, with each fraction containing from 9 to 15 peaks Because the retention times between each fraction could not be compared, it was felt that to collect pure components from these fractions would involve a great deal of repetition. An infrared spectrum of each of the fractions indicated that there was no separation into groups of components with common functional groups; each fraction exhibited carbonyl absorption. The infrared spectra also indicated that each fraction contained alcoholic or phenolic moities.

The next attempt to fractionate the oil was by using a "spinning band" distillation column. Eighteen fractions were collected (Table 2). On submitting these fractions to GLC it was found that there was a great deal of overlapping between fractions. It was also observed that the vacuum trap contained 3-5 ml of a very fragrant oil. It was therefore feared that many of the more volatile components were being lost in the vacuum system.

At this point it was decided to use gas chromatography throughout this investigation as the best means of separation and collection of pure components of Canadian snake-root oil. It was felt that this method would be the least harmful to the oil, and that no components would be lost in the fractionation procedure.

An Apiezon L column became available at this point in the study. By varying the volume of the injection and the gas chromatograph operating conditions, the number of peaks resolved eventually reached 25, most of which were well defined (Tables 1 and 5). This compares with 13 components resolved by Bauer employing gas chromatography.

In order to collect and identify each component, a preliminary fractionation of the oil into five fractions was attempted and accomplished using a Varian Aerograph model 712 preparative gas chromatograph (Figure 1). The majority of the components formed aerosols as they emerged from the column and this made collection relatively inefficient. Steel wool scrubbing material (Kurly Kate) was placed in the collection bottles to aid the collection, however results were not greatly improved.

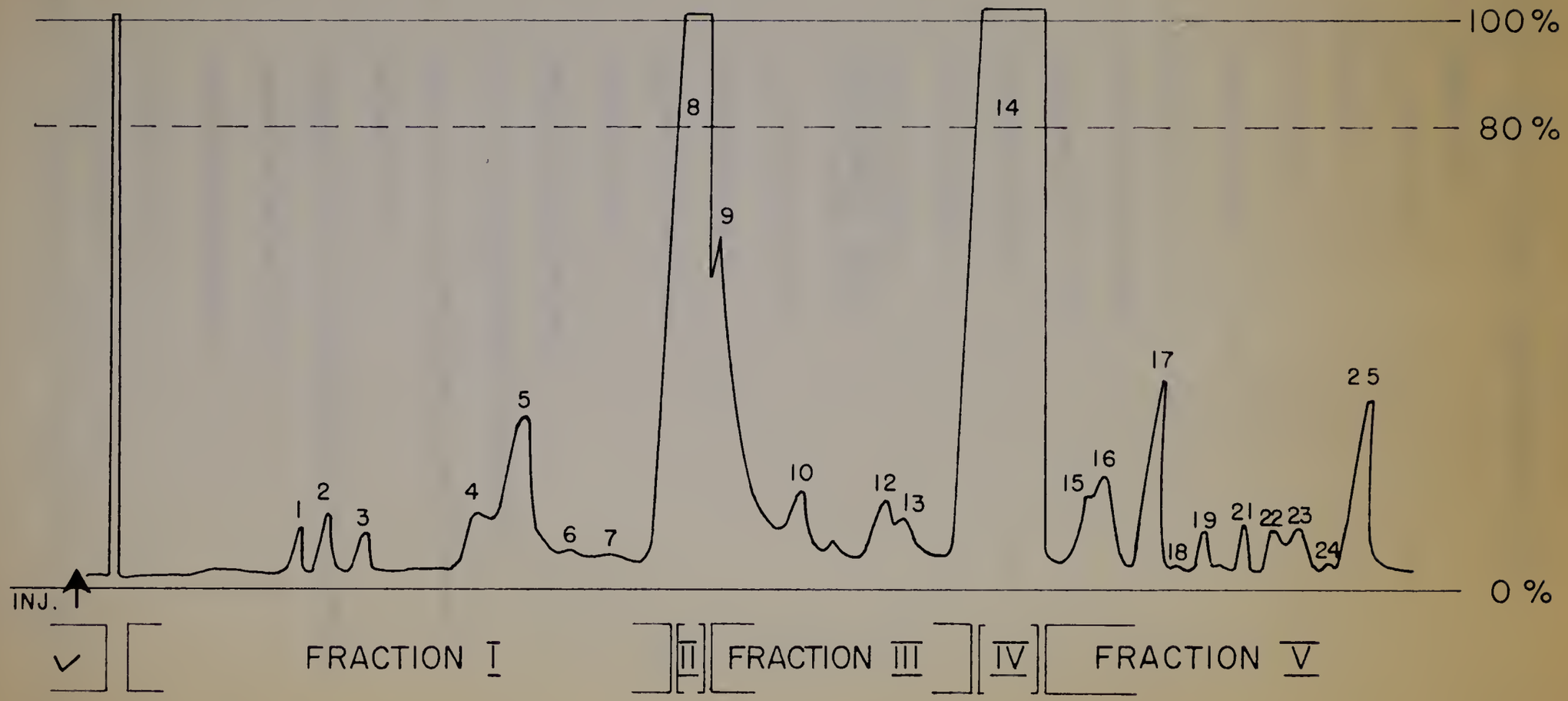


Figure 1. Gas Chromatogram of Oil of Canadian Snake-root (Sample A) Using the 20 Foot Apiezon L Column

Table 1

Composition of Oil of *Asarum Canadense* Sample A

Peak No.	Compound*	%
1	(β-ocimene)	1.23
2	myrcene	1.45
3	β-pinene	1.02
4	unidentified	1.30
5	linalool	6.83
6	unidentified alcohol	trace
7	unidentified alcohol	trace
8	linalyl acetate	27.59
9	α-terpineol	5.91
10	bornyl acetate	1.81
11	unidentified ester	1.94
12	unidentified ester	3.04
13	unidentified ester	1.89
14	methyleugenol	30.48
15	*cis*-methylisoeugenol	1.75
16	unidentified	
17	*trans*-methylisoeugenol	6.62
18	(2,3,4,5-tetramethoxyallylbenzene)	0.37
19	(*cis*-2,3,4,5 tetramethoxypropenylbenzene)	0.46
20	unidentified	trace
21	(*trans*-2,3,4,5-tetramethoxypropenylbenzene)	1.45
22	unidentified alcohol	1.30
23	unidentified alcohol	1.26
24	unidentified	trace
25	(aristolone)	2.30

* Names in parentheses refer to tentatively identified compounds

Cooling of the collection bottles did not appear to increase collection efficiency in the case of the eluates which formed aerosols, but did help in collection of the very volatile components. It is possible that by cooling the collection bottle too much, water will condense in the collection bottle.

Because of the difficulties of collection, the preliminary separation was very time consuming, with collection of approximately 5-10 ml of each fraction taking many weeks of almost continous operation of the gas chromatograph.

An F and M model 500 gas chromatograph was employed for the separation of each fraction into pure components. The best method of collecting small quantities of pure components for identification purposes was to use an open-ended melting-point capillary tube inserted through a hole in a rubber septum installed at the exit tip of the chromatograph. Every effort was made to obtain as pure a sample as possible, and to this end "heart cutting" of each peak was employed, and the homogeneity of each component was checked by thin layer chromatography. Enough pure sample of each component was collected for infrared and mass spectral analysis, and in some cases for an ultraviolet spectrum and refractive index determination. Many of the emerging components formed aerosols, as was observed with the initial fractionation on the Varian Aerograph instrument, so that collection efficiency was quite low. The collection of components one to five, which were quite volatile, was facilitated by wrapping a filter paper wick soaked in methylene chloride around the collection capillary.

The remaining components were collected in capillaries at room temperature.

When collecting a sample for mass spectrometry enough runs were done to deposit approximately 5 mg of sample in the tube. The ends of the capillary tube were then sealed and the sample submitted for mass spectral analysis. Many minor components required a great many runs before enough pure sample was collected for infrared or mass spectral analysis.

The results and conclusion made for each component will be discussed separately for each component.

Component 1

Gas chromatographic retention times of a variety of common terpenes and related compounds were recorded but the retention time of Component 1 did not match any of them. Therefore a tentative identification was not possible until the mass and infrared spectra were recorded.

The mass spectrum showed a parent peak of m/e 136. An accurate mass determination of this ion was made which indicated a formula of $C_{10}H_{16}$. This formula confirms unsaturation, and indicates the presence of three double bonds and/or rings. It was therefore likely that component 1 was a monoterpene hydrocarbon.

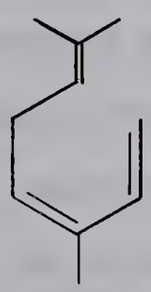
Comparing the mass spectrum with those spectra of monoterpene hydrocarbons published by Ryhage (20) it was seen that it matched the spectrum of β-ocimene-X.

β-Ocimene-X (XXIV) is a monoterpene hydrocarbon of molecular weight 136 ($C_{10}H_{16}$) with three double bonds in the molecule, two of which are in conjugation. The mass spectra of β-ocimene-X and β-ocimene-Y (XXV) were very similar, the only significant difference between them being the intensity of the peak at m/e 80 compared with the surrounding peaks. In the spectrum of the Y form the peak at m/e 80 was much more abundant than the peaks surrounding it. In the published spectrum of the X form the peak at m/e 80 was similar in intensity to the surrounding peaks. The spectrum of component 1 showed a peak at m/e 80 of similar abundance to the surrounding peaks. Therefore

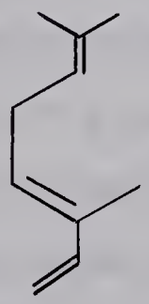
if component 1 was ocimene it is likely that it was the X form and not the Y form.

Component 1 was present in Canadian snake-root oil in small quantity; this, together with its volatility made collection difficult and time consuming. Due to insufficient sample being collected the infrared spectrum was weak and poorly resolved. Only two peaks of appreciable intensity were observed.

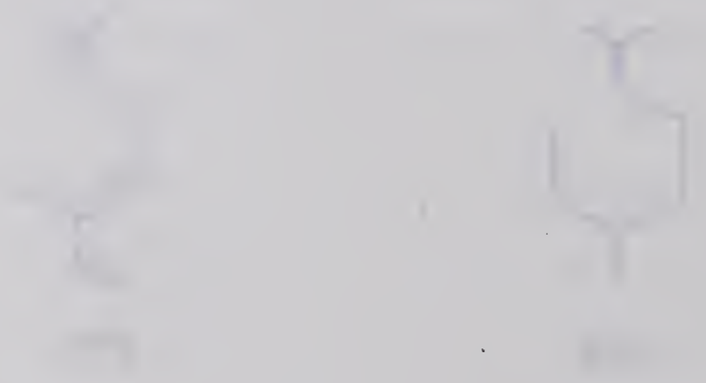
On the basis of the spectral evidence, a tentative identification of component 1 can be made as β-ocimene-X. This compound has not been reported present in the oils of any Asarum species. It has been reported present in many other plants including Ocimum basilicum, Homoranthus flavescens and Myrtaceae species (39).



XXIV



XXV

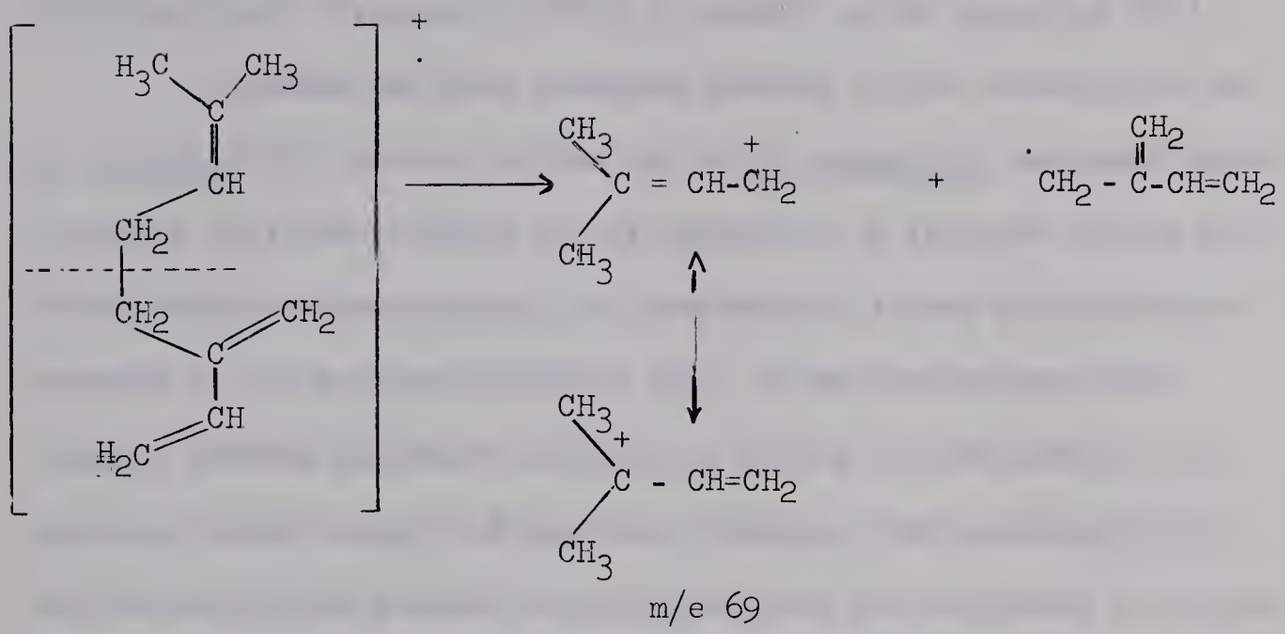


Component 2

The retention times for component 2 were the same as those recorded under identical conditions for an authentic sample of myrcene on two different columns.

Myrcene is an acyclic monoterpene hydrocarbon of molecular weight 136 ($C_{10}H_{16}$). Its mass spectrum was included in those monoterpene hydrocarbons published by Ryhage (20), and the mass spectrum of component 2 was compared with it. The two spectra were identical except the published base peak was at m/e 41 and the peak at m/e 93 was 85%, while the base peak for component 2 was m/e 93 and the abundance of m/e 41 was 87.5%. This slight change of abundances could be due to the fact that different instruments were used to record the two spectra. As stated by Budzikiewicz "substantial differences may be encountered in spectra measured on instruments of varying designs" (40). In most instances the differences are only of a quantitative nature, as is the case with the spectra of myrcene and component 2.

The three most abundant peaks in the spectrum were at m/e 93, m/e 69, and m/e 41. The base peak at m/e 93 was due to the loss of a C_3H_7 radical from the molecular ion, which was confirmed by the metastable peak observed at m/e 63.8. The peak at m/e 69 was the result of allylic cleavage between the two isoprene units in the molecule (20).



This cleavage agrees with the general rule for predicting prominent peaks that double bonds favor allylic cleavage and give the resonance-stabilized allylic carbonium ion -- (41). The peak at m/e 41 was due to the loss of C_2H_4 from m/e 69, a fragmentation which was confirmed by the presence of a metastable peak at m/e 24.3.

The infrared spectrum of component 2 was superimposable upon the spectrum of an authentic sample of myrcene. (The differences in spectra as quoted in the experimental section are more apparent than real. The differences arise from measurement of the peak in wavelength units (microns) and then converting this figure to wavenumber (cm^{-1}). When the spectra of the component and the authentic sample are placed together they are superimposable.) A large band was seen at 889 cm^{-1} which is attributable to C-H bending vibrations of a 2,2-disubstituted vinyl structure ($\geq C=CH_2$) (42). This structure is present in the myrcene molecule. Two bands were observed at 986 and 903 cm^{-1} which are likely due to C-H bending vibrations of the monosub-

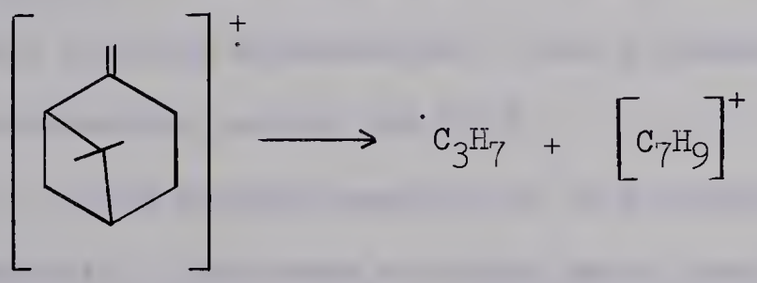
stituted vinyl structure ($-CH=CH_2$) present in the molecule (42).

Myrcene has been reported present in the volatile oil of A. europeum (2), but not in the oil of A. canadense, although Bauer reported (19) the presence of β-myrcene in a fraction of the oil after column chromatography, but stated that it was definitely not present in the original volatile oil. It was determined that linalyl acetate underwent elimination during chromatography on an alumina column to give β-myrcene, linalool, and unchanged ester. As the oil in the present investigation was not subjected to column chromatography prior to collection of pure components, it can be concluded that myrcene was present as such in the original oil. It might be argued that elimination of linalyl acetate could also occur during passage through a gas chromatographic column, and it is its elimination products which emerge from the column and are collected as pure components. However, this possibility was discounted when a pure sample of linalyl acetate was chromatographed (GLC) under conditions identical with the collection of myrcene. There was only one peak recorded and no peak corresponding to myrcene was observed.

Component 3

The retention time (GLC) of component 3 matched the time recorded for an authentic sample of β-pinene. Pinene was reported present in Canadian snake-root oil by Power (18), but could not be found in the sample of oil investigated by Bauer (19), although attempts were made to find this hydrocarbon before and after column chromatography of the oil.

With a tentative identification of component 3 as β-pinene in hand, its mass spectrum was compared with the published spectra of monoterpene hydrocarbons (20). The spectrum of component 3 and the spectrum of β-pinene were identical. The base peak at m/e 93 is formed by the loss of the isopropyl group from the molecular ion (XXVI) which is



XXVI

confirmed by a metastable peak at m/e 63.8. The fragment of m/e 93 can also be formed by a two step reaction, m/e 136 ⟶ me/ 121 ⟶ m/e 93 (20) (confirmed by metastable peaks at m/e 107.5 and m/e 71.5).

The infrared spectrum of component 3 was superimposable on a spectrum of an authentic sample of β-pinene. A doublet of medium intensity was observed at 1380 and 1365 cm^{-1} due to C-H

bending vibration of a gem-dimethyl group. A strong band attributable to C-H bending vibrations of the 2,2-disubstituted vinyl structure ($\rangle C=CH_2$) was observed at 875 cm^{-1}.

Component 4

This compound was not identified. Its mass spectrum was similar to the published spectrum of p-cymene (20), but a comparison of its infrared spectrum with that of an authentic sample of p-cymene proved that it was not this compound. An accurate mass determination of the molecular ion m/e 134 indicated the formula $C_{10}H_{14}$. This suggested that it was an unsaturated monoterpene with four double bonds and/or rings. The presence of an abundant parent peak suggested that the compound was aromatic. The base peak at m/e 119 was formed by the loss of methyl radical from the molecular ion. Such a fragmentation was supported by a metastable peak at m/e 105.8.

The infrared spectrum of this compound indicated non-aromaticity. There were no strong bands observed in the 1610 to 1480 cm^{-1} region where skeletal vibrations of aromatic rings occur (43). In addition there were no bands below 813 cm^{-1} which would be attributable to C-H out of plane bending and benzene ring substitution.

Component 5

The retention time (GLC) of component 5 on two different columns matched the retention time recorded for an authentic sample of linalool (XVI). As linalool is a known component of the oil of Canadian snake-root a mass spectrum was not recorded.

An infrared spectrum of component 5 was identical to that of an authentic sample of linalool. It showed O-H stretching at 3340 cm^{-1} and the two bands at 1400 and 1363 cm^{-1} can be assigned to O-H bending. The strong band at 1105 cm^{-1} is attributed to the C-O stretching of the tertiary alcohol group.

A refractive index was determined for component 5 and agreed with the literature value for linalool (39).

Component 6

The gas chromatographic retention time of component 6 did not match the retention times recorded for the authentic samples available in this study. Its mass spectrum indicated a molecular weight of 154. An accurate mass determination of the m/e 154 ion confirmed the formula $C_{10}H_{18}O$. Because two ions were observed at m/e 139 $(M-15)^+$ and m/e 136 $(M-H_2O)^+$ it was considered likely that component 6 was an alcohol with a molecular ion of 154 (21). Comparing the mass spectrum with those published by von Sydow (21) for monoterpene alcohols it was seen that it matched the published spectrum of fenchyl alcohol.

An infrared spectrum was recorded for component 6. This spectrum was consistent with an alcohol in that an O-H stretching

vibration band was observed at 3330 cm^{-1}, and another was observed at 1123 cm^{-1} which indicated that the compound might be a tertiary alcohol. It was suspected, therefore, that compound 6 was not fenchyl alcohol which is a secondary alcohol. This was confirmed by recording an infrared spectrum of an authentic sample of fenchyl alcohol. The two spectra were quite dissimilar. An infrared spectrum was also recorded for β-terpineol and isopulegol, but neither of them matched the spectrum of component 6.

Component 7

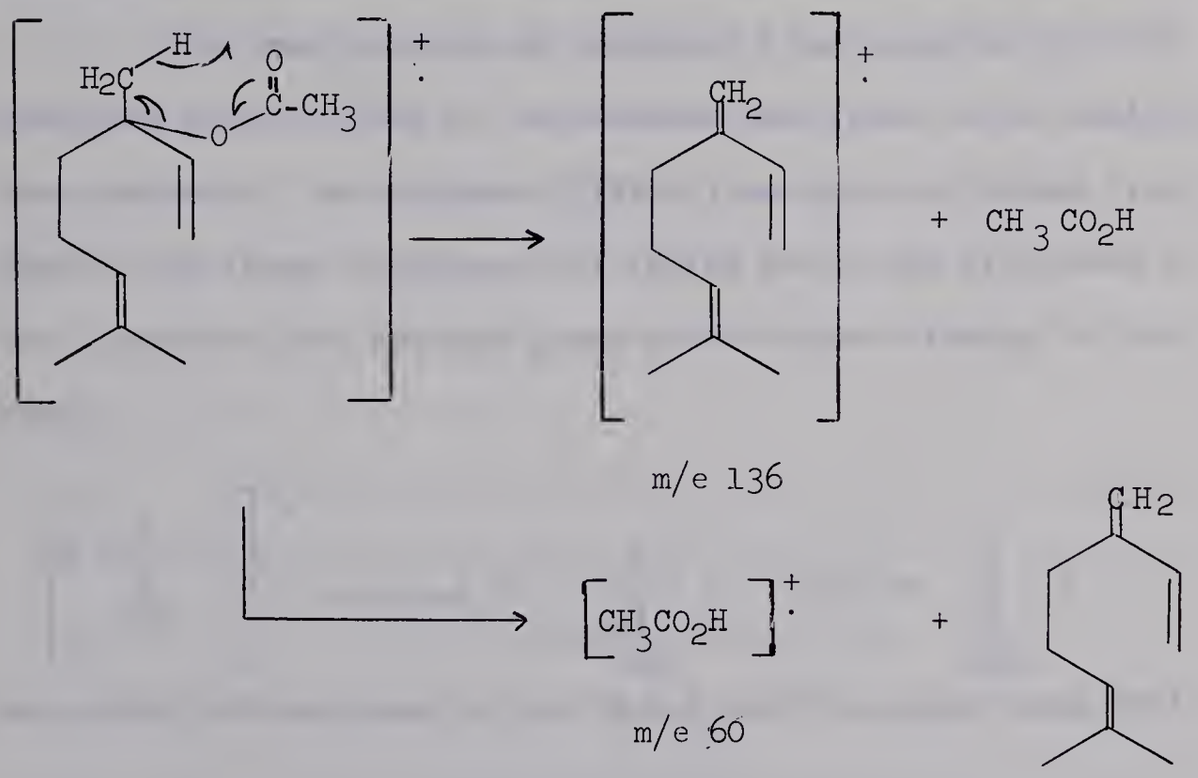
Canadian snake-root oil contained only trace amounts of compound 7. Its retention time did not match the retention times recorded for the authentic samples available in this study.

The mass spectrum for this compound showed a parent peak at m/e 152, and an accurate mass determination of this ion indicated a formula $C_{10}H_{16}O$, which suggested a molecule with a molecular weight of 152 containing three double bonds and/or rings. Two weak peaks were observed in the mass spectrum at m/e 137 $(M-15)^+$ and m/e 134 $(M-18)^+$ which suggested that this compound was a monoterpene alcohol, as the presence of the hydroxyl group is always revealed by the occurence of an $(M-18)^+$ ion (21).

The infrared spectrum of component 7 supported the possibility of this compound being an alcohol. A large band was observed at 3380 cm^{-1} due to absorption by the hydroxyl group; the low frequency of this band due to intermolecular bonding (43). Two bands at 1370 and 1148 cm^{-1} suggested that the alcohol was tertiary.

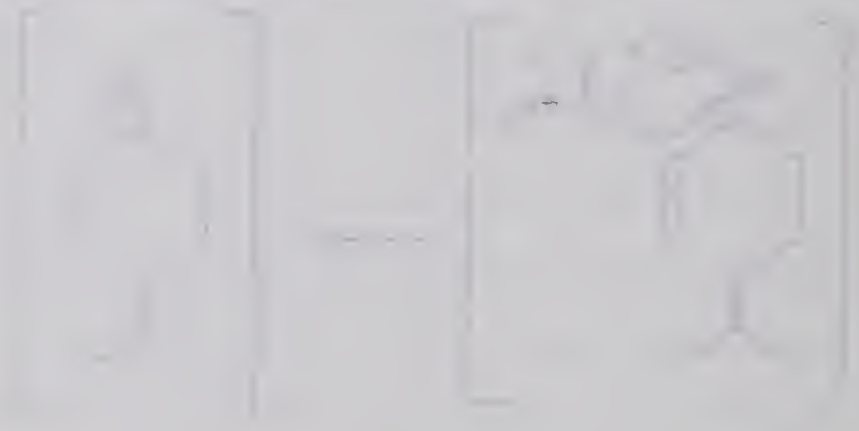
Component 8

This component had the same retention time (GLC) as a pure sample of linalyl acetate on two different columns. The mass spectrum matched the published spectrum of linalyl acetate (19). Like many terpene esters it lacked the molecular ion peak at m/e 196 (19,22), and the first fragment was observed at m/e 136 $(M-60)^+$, due to the elimination of acetic acid by a McLafferty rearrangement except that the charge remains with the olefinic rather than the carbonyl-containing moiety (44). A peak at m/e 60 was observed which is attributed to acetic acid; a reaction which may be visualized as a simple McLafferty rearrangement (XXVII).



XXVII

A final confirmation of the identity of component 8 was its infrared spectrum which was superimposable on a spectrum of an authentic sample of linalyl acetate. Two bands at 1740 and 1240 cm^{-1} caused by C=O stretching and C-O stretching respectively, were



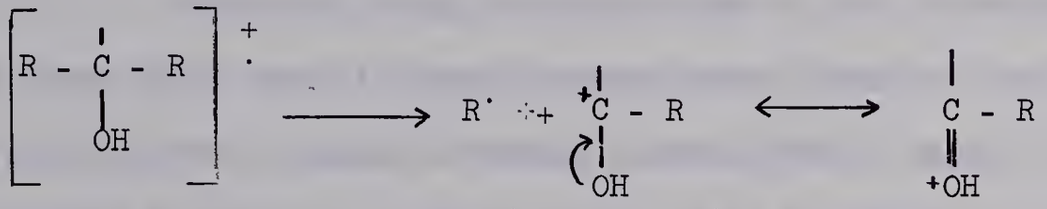
prominent in the infrared spectrum, and are characteristic of acetates (45).

Linalyl acetate has been reported present in the volatile oil of A. canadense (19). Bauer reported that it constituted 41.1% of the oil. In the present investigation linalyl acetate constituted 27.59% of sample A.

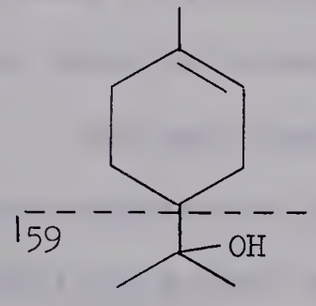
Component 9

The retention time (GLC) of component 9 corresponded to the retention time recorded for a pure sample of α-terpineol, which is a known constituent of Canadian snake-root oil (19).

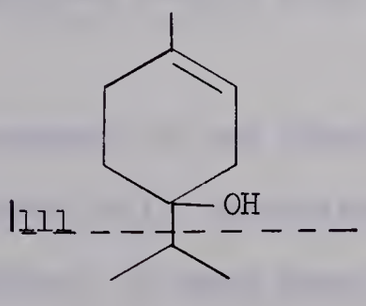
The mass spectrum of component 9 was compared with the published spectrum (21) of α-terpineol and found to be identical. The spectrum of α-terpineol (XXVIII) was quite different from that of its isomer terpinene-4-ol (XXIX) due to the difference in the location of the hydroxyl group which induces cleavage of the type:



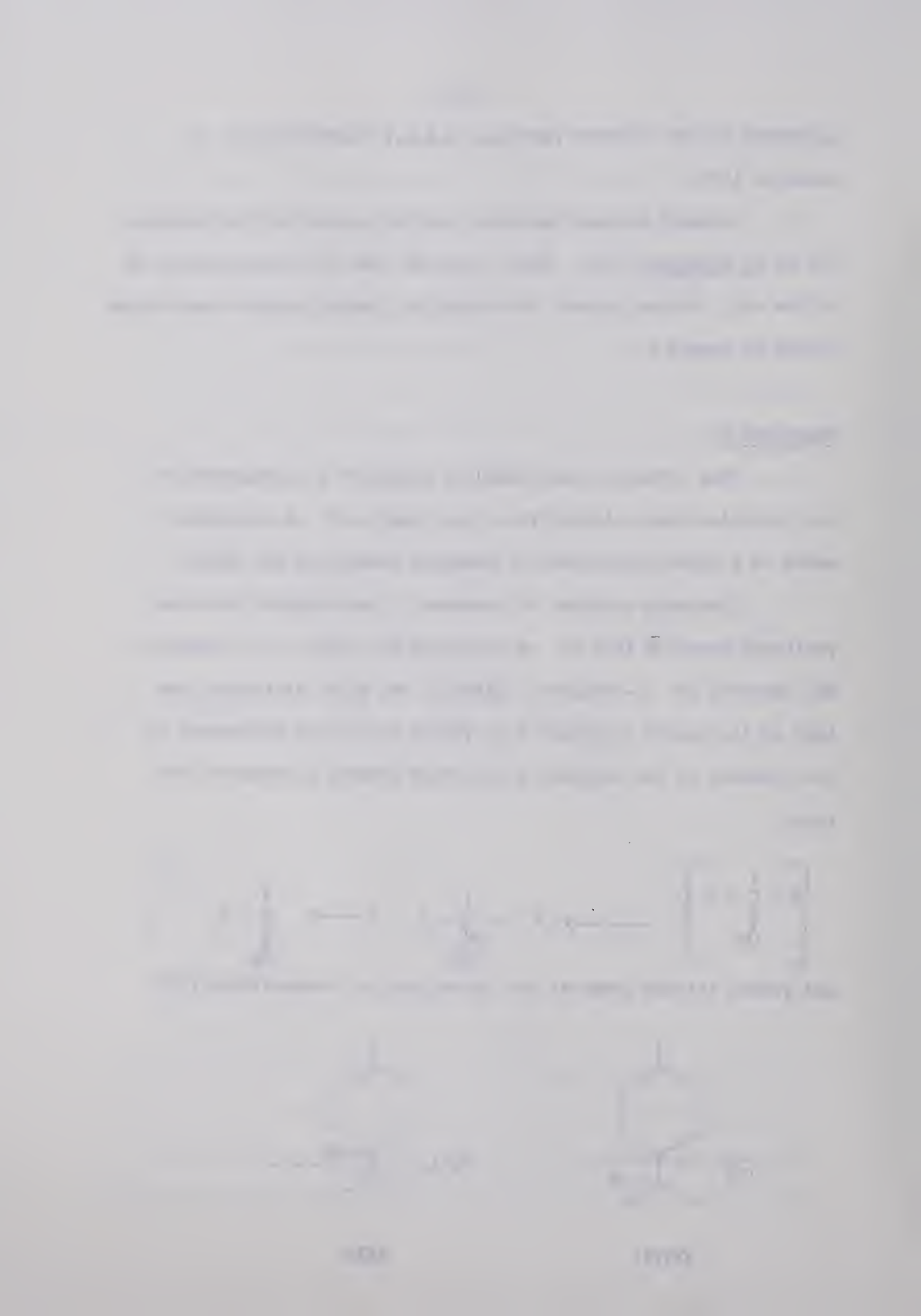
and yields intense peaks at m/e 59 and m/e 111 respectively (46)



XXVIII



XXIX



The base peak of m/e 59 for α- terpineol is characteristic of this compound due to the unique attachment of the hydroxyl group to the isopropyl group (21).

The mass spectrum is a good illustration of the molecular ion peak being absent for some monoterpene alcohols. The occurrence of peaks at m/e 139 $(M-15)^+$ and m/e 136 $(M-18)^+$ simultaneously, made it possible to calculate the molecular ion, as a result of the observation made by von Sydow (21).

The infrared spectrum of component 9 was identical to the spectrum of an authentic sample of α-terpineol. Absorption due to O-H stretching was observed at 3340 cm^{-1}, and a band at 1156 cm^{-1} was due to the O-H bending of a tertiary alcohol A band of medium intensity was observed at 834 cm^{-1} which has been attributed to the C=C bending of the trisubstituted double bond (47).

Component 10

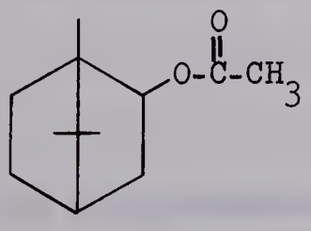
Component 10 had retention times on two chromatographic columns (GLC) equal to those recorded under identical conditions for an authentic sample of bornyl acetate (XXX). Bauer (19) has reported the presence of bornyl acetate in Canadian snake-root oil, while Power (18) identified the alcohol borneol from the sample of the oil which he investigated.

The mass spectrum of component 10 was identical to the published spectrum of bornyl acetate (22). The molecular ion (m/e 196) was present in the spectrum. A large peak was observed

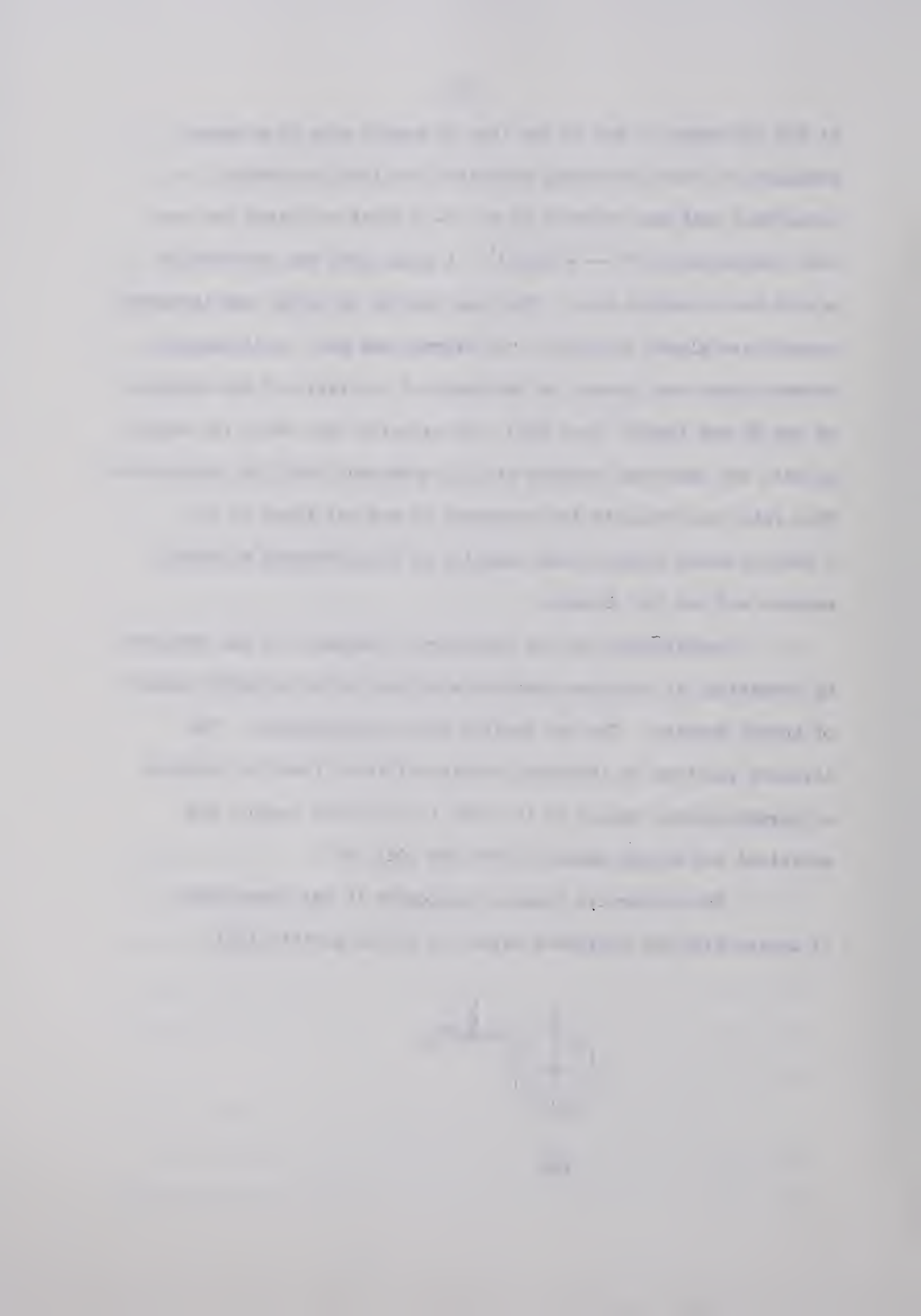
at m/e 136 which is due to the loss of acetic acid in a manner analagous to that previously described for linalyl acetate. A metastable peak was observed at m/e 94.3 which confirmed the one-step fragmentation $M^+ \longrightarrow (M-60)^+$. A small peak was observed at m/e 60 due to acetic acid. The mass spectra of bornyl and isobornyl acetate are almost identical, but Biemann was able to distinguish between these two isomers on the basis of the ratio of the abundance of the M^+ and $(M-60)^+$ ions (48). He reported this ratio for bornyl acetate and isobornyl acetate as 0.31:5.08 and 0.04:5.94 respectively. This ratio was measured for component 10 and was found to be 0 46:5.15 which supports the identity of this compound as bornyl acetate and not its isomer.

Confirmation of the identity of component 10 was obtained by comparing its infrared spectrum with that of an authentic sample of bornyl acetate. The two spectra were superimposable. The infrared spectrum of isobornyl acetate differed from the spectrum of bornyl acetate mainly in the 1000 to 1100 cm^{-1} region, and exhibited two strong bands at 1057 and 1021 cm^{-1}.

The refractive index of component 10 was determined. It agreed with the published value for bornyl acetate (39).



XXX



Component 11

The retention time (GLC) of component 11 did not match any of those recorded for known constituents for Canadian snake-root oil. The infrared spectrum indicated the possibility of an ester, as a strong band was observed at 1730 cm^{-1} due to carbonyl absorption. A band due to the C-O stretching was observed at 1232 cm^{-1}, which frequency could indicate that the compound was an acetate, as the region for the C-O stretching of an acetate is near 1245 cm^{-1} (45).

With the infrared evidence that component 11 was an ester, its mass spectrum was compared with the published (22) spectra of esters of monterpene alcohols. Like many terpene esters the molecular ion peak was absent, and the first significant peak was observed at m/e 136. Two small peaks were observed at m/e 60 and m/e 61 corresponding to acetic acid and $(CH_3COOH_2)^+$ respectively (22). Thus the available evidence indicates that component 11 is a terpene ester, probably an acetate. The mass spectrum of component 11 was similar to the published spectrum of geranyl acetate. However they were not identical. The presence of geranyl acetate in A. canadense is not unexpected as the parent alcohol, geraniol, was found both by Power and Bauer. The presence of free geraniol was not detected in this investigation.

Component 12

Component 12, like component 11, remains unidentified. Its infrared spectrum contained a strong peak at 1725 cm^{-1} due to carbonyl stretching vibrations and a strong, very broad band at

1260 to 1230 cm^{-1} due to C-O stretching. Both bands are consistent with the component being an ester. The broad nature of the band at 1260 to 1230 cm^{-1} could indicate some impurity present in the sample. Two strong bands were observed at 1380 and 1360 cm^{-1}, suggestive of the C-H bending of a gem-dimethyl group.

The mass spectrum was almost identical to the published spectrum of linalyl acetate (19). This suggested that the hydrocarbon skeleton of the molecule was isomeric with linalyl acetate. A peak was observed at m/e 60 which could be attributed to acetic acid; thus component 12 could be an acetate.

Component 13

Hegnauer (49) has made an unsubstantiated claim for the presence of eugenol in the volatile oil of A. canadense contrary to the findings of the two prime investigators, Power (18) and Bauer (19), of this plant, neither of whom claim eugenol as a constituent of Canadian snake-root oil. This contradiction has now been resolved.

The identity of component 13 as eugenol (IV) was first suspected because of the distinctive odor of the vapor as it emerged from the GLC column.

The mass spectrum of component 13 confirmed that the compound had a molecular weight of 164, and an accurate mass determination of the m/e 164 ion confirmed the formula $C_{10}H_{12}O_2$. The parent peak was the base peak which is often observed in mass spectra of phenols due to the stability conferred by the benzene ring.

The identity of component 13 as eugenol was confirmed by comparing the infrared spectrum with that of an authentic sample of eugenol. The two spectra were superimposable. Three bands are present in the infrared spectra of phenols which result from O-H stretching, C-H stretching and O-H bending (43). In the spectrum of eugenol the band due to O-H stretching was observed at 3500 cm^{-1}. A strong band due to the C-O stretching was observed at 1150 cm^{-1}. A band was observed at 747 cm^{-1} due to out-of-plane bending of the bonded OH group. Naves (50) has reported that bands due to deformation vibrations of C-H at 996 cm^{-1} and 917 cm^{-1} are characteristic for eugenol. The first band is ascribed to the -CH=C< group and the second to the >C=CH_2 group. In the infrared spectrum of component 13 these two bands were observed at 998 and 920 cm^{-1}.

Component 14

Component 14 was the most abundant constituent of the oil of Canadian snake-root. As Power (18) reported that the oil contained mainly methyleugenol, and Bauer (19) reported it present in the amount of 44.5%, it was felt that component 14 would be methyleugenol. The gas chromatographic retention times matched those of an authentic sample of methyleugenol on two different columns. The mass spectrum was identical to the published spectrum of methyleugenol (19). A peak was observed at m/e 151 which Bauer reported was the distinguishing feature between the spectra of methyleugenol and methylisoeugenol as this ion was absent in the latter spectrum (19).

The infrared spectrum of component 14 was superimposable upon the spectrum of an authentic sample of methyleugenol. A number of strong bands were observed in the 1250 to 1059 cm^{-1} region, which would be attributed to the asymmetrical C-O-C stretching vibrations. Two strong bands were observed at 908 and 840 cm^{-1} which were attributed to the olefinic C-H out-of-plane, in-phase bending vibrations (42).

The ultraviolet spectrum was recorded for component 14 and the recorded maxima were in agreement with the literature values (51).

Component 15

Component 15 has been identified as cis-methylisoeugenol. This compound has not been previously reported for A. canadense. Its mass spectrum was almost identical to that of methyleugenol except that a peak at m/e 151, which is present in the spectrum of the latter, was absent. This fact has been noted previously by Bauer (19). The parent peak, which was also the base peak, was at m/e 178. An accurate mass determination of this ion confirmed the formula $C_{11}H_{14}O_2$. An abundant peak was observed at m/e 163, due to the loss of a methyl radical from the molecular ion. This fragmentation was confirmed by the presence of a metastable ion of m/e 149.3.

The gas chromatographic retention time relative to 1,2,3-trimethoxybenzene was measured as 2.05 which was in agreement with the literature (52) value.

As it was necessary to have an authentic sample of cis-methylisoeugenol for comparison, a sample of methylisoeugenol (mixture of cis and trans isomers) was purchased commercially.

It is known that the two isomers can be separated by gas chromatography and that the cis isomer will always precede the trans isomer in order of emergence from the column irrespective of the relative polarity of the chromatographic column (52,53). The sample of methylisoeugenol was chromatographed, and two pure components were collected, which by their order of emergence and infrared spectrum (50,53), corresponded to cis and trans-methylisoeugenol.

An ultraviolet spectrum of component 15 was recorded in aqueous ethanol. An absorption maximum was observed at 255 mμ. Mahboob (51) reported that the absorption maximum of methylisoeugenol was located at 258 mμ which apparently was a value for a mixture of the cis and trans isomers of this compound. Hunakubo (54) reported that the absorption maxima of cis-methylisoeugenol were present at 228 and 280 mμ.

The figure of 228 mμ reported by Hunakubo for the primary band of cis-methylisoeugenol appears to be in error. The absorption of the primary band of methyleugenol is located at 230 mμ (51). As this molecule has no extended conjugation it seemed that the absorption of the primary band of cis-methyleugenol, which has extended conjugation into the propenyl side-chain, should be somewhat greater. This contradiction has been resolved by recording a spectrum of an authentic sample of cis-methylisoeugenol. An absorption maximum was located at 255 mμ.

The infrared spectrum furnished further evidence that component 15 was the cis and not the trans form of methylisoeugenol. As reported by Naves (50), the cis and trans forms of propenylbenzenes can be differentiated by means of the C-H deformation vibrations of the -CH=CH- group. Cis compounds absorb around 710 cm^{-1} in the infrared and the trans form around 965 cm^{-1}. Naves reported the values for cis and trans-methylisoeugenol as 715 and 967 cm^{-1}. The infrared spectrum of component 15 was transparent in the region of 965 cm^{-1}, but there was a weak absorption band observed at 710 cm^{-1} typical of the cis configuration. To confirm the identity of component 15, an infrared spectrum of an authentic sample of cis-methylisoeugenol was recorded. It was superimposable on the spectrum of component 15.

Cis-methylisoeugenol has not been reported as a constituent of Canadian snake-root oil. Hegnauer (49) reported methylisoeugenol as a constituent of the volatile oil of A. arifolium, but he did not indicate whether it was the cis or trans isomer.

Component 16

Component 16 emerged from the GLC column partially overlapped with component 15. Because of this, very little of the pure compound was collected. The infrared spectrum was not inconsistent with an α,β-unsaturated ketone; a strong absorption band was present at 1682 cm^{-1}. There was not sufficient sample available for any chemical tests to be performed to confirm that this component was, in fact, a ketone.

The mass spectrum was very similar to the published spectrum of aristolone (19). A parent peak was observed at m/e 218, and an abundant fragmentation peak was observed at m/e 189 due to the loss of C_2H_5 from the molecular ion (a metastable peak was observed at m/e 164 which confirmed this fragmentation). The possibility exists that component 16 is an isomer of aristolone.

Component 17

The mass, infrared, and ultraviolet spectral data for component 17 were quite similar to the data described previously for cis-methylisoeugenol. It was suspected, therefore, that the compound was the trans isomer of methylisoeugenol. Therefore features which identify a trans compound were looked for. The infrared spectrum exhibited a strong absorption band at 960 cm^{-1} characteristic of a trans configuration of the -CH=CH- group (50). The ultraviolet spectrum showed the expected bathochromic shift of a trans compound compared with the cis isomer (51,54). The trans compound was eluted after the cis compound from the GLC column which is in agreement with the behavior of cis and trans-propenyl isomers (52). The relative retention time was measured as 2.74, consistent with the literature (52) value for trans-methylisoeugenol.

The identity of component 17 as trans-methylisoeugenol was confirmed by comparing its infrared spectrum to the spectrum of an authentic sample (obtained as previously described) of trans-methylisoeugenol. The two spectra were identical.

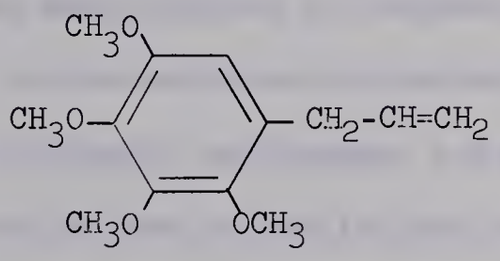
Trans-methylisoeugenol has not been reported before as a constituent of Canadian snake-root oil.

Component 18

The relative retention time of component 18 agreed with the literature (52) value for 2,3,4,5-tetramethoxyallylbenzene (XXXI). Bauer had found this compound in a minute quantity in Canadian snakeroot oil (19). He identified it by its mass and NMR spectra.

The mass spectrum of component 18 differed from the spectrum published by Bauer (19) in the relative intensities of the peaks. The parent peak at m/e 238 was not the base peak as was the case in the published spectrum.

The ultraviolet spectrum, with maxima at 228 and 282 mμ was consistent with the proposed structure. These maxima are almost identical to those found for methyleugenol which was expected as the two additional methoxy groups on the molecule should not have much effect on the position of the primary band.

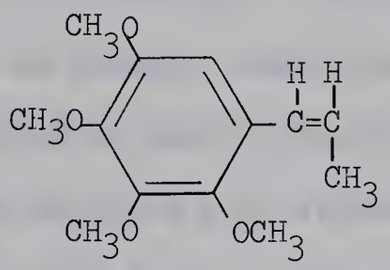


XXXI

Because of insufficient sample, an infrared spectrum of component 18 was not recorded. Thus on the basis of relative retention time and the ultraviolet spectrum, only a tentative identification of component 18 as 2,3,4,5-tetramethoxyallylbenzene can be made. An authentic sample was not available to record a mass spectrum for comparison with the spectrum of component 18.

Component 19

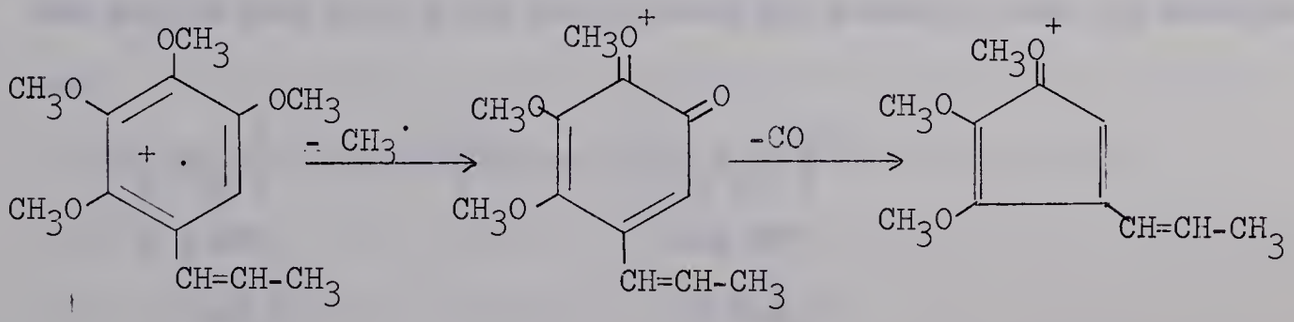
The relative retention time of component 19 agreed with the literature (52) value for cis-2,3,4,5-tetramethoxypropenylbenzene (XXXII).



XXXII

Although Bauer had found the allyl form of this molecule in A. canadense and Guenther (55) reported that allyltetramethoxybenzene was isolated from French parsley seed oil, no reference could be found that the propenyltetramethoxybenzenes are present in essentials oils.

The mass spectrum of component 19 showed a parent peak at m/e 238. An accurate mass determination of this ion confirmed the formula $C_{13}H_{18}O_4$. An abundant ion was observed at m/e 208 $(M-CH_2O)^+$, and another at m/e 193 due to loss of a methyl radical from the fragment of mass 208 (confirmed by a metastable peak at m/e 179). Small peaks were observed at m/e 223 $(M-15)^+$ and m/e 195 $(M-15-28)^+$ (XXXIII).



m/e 238 m/e 223 m/e 195

XXXIII

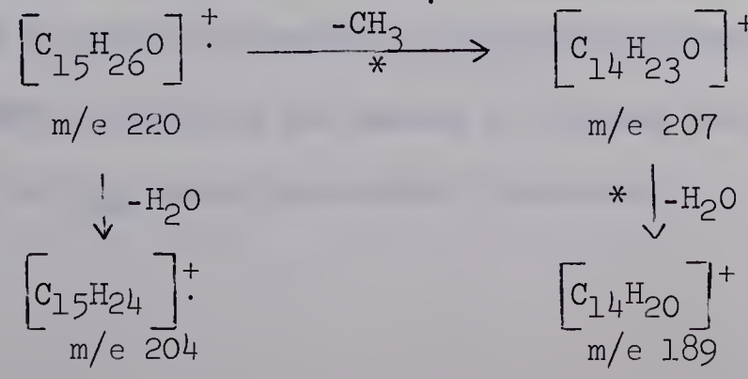
This fragmentation is reminiscent of that described by Barnes et al (56) for pyrogallol trimethyl ether and of methyleugenol as described by Bauer (19).

The infrared spectrum was consistent with the proposed structure for this component. A strong band due to C-H bending of one hydrogen in an aromatic system was observed at 885 cm^{-1}. Four bands were observed at 1640, 1585, 1495, and 1460 cm^{-1} indicative of carbon-carbon multiple bond stretching in an aromatic ring. Absorption due to the C-O stretching of the methoxy group was observed by a strong peak at 1235 cm^{-1}.

An authentic sample of cis-2,3,4,5-tetramethoxypropenylbenzene was not available for comparison.

Component 20

Component 20 was not identified. The mass spectrum had a parent peak of m/e 222. An accurate mass determination of this ion indicated the formula $C_{15}H_{26}O$. The base peak was m/e 207, due to the loss of a methyl radical from the molecular ion. This fragmentation was confirmed by the metastable ion at m/e 193.1. A large ion was observed at m/e 189. A metastable peak at m/e 172.6 indicated that it was due to the loss of H_2O from the ion of mass 207. There was also a peak at m/e 204 due to loss H_2O directly from the molecular ion.

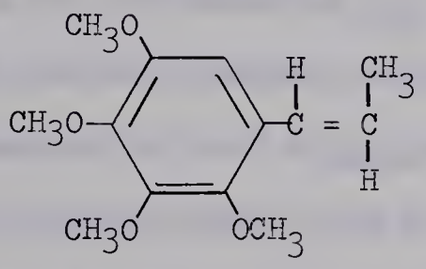


The infrared spectrum exhibits a doublet at 1375 and 1365 cm^{-1} which is the characteristic region for the C-H bending of a gem-dimethyl group. Two bands attributed to C-H bending vibrations of a methyl group were observed at 1375 and 1455 cm^{-1} . A band was observed at 1255 cm^{-1} characteristic of the C-O-C stretching vibrations of an ether.

Component 20 was not identified because of insufficient sample and time.

Component 21

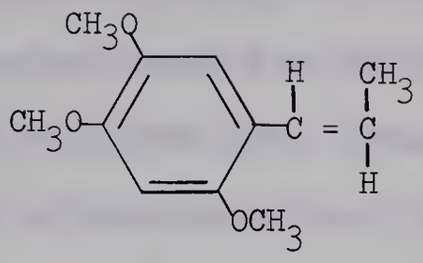
The relative retention time of component 21 was measured as 4.93 which was close to the published (52) relative retention time of trans-2,3,4,5-tetramethoxypropenylbenzene (XXXIV).



XXXIV

This compound has not been previously reported present in A. canadense.

The ultraviolet spectrum of this component exhibited the maxima expected for such a structure. Two maxima were observed at 260 and 302 mμ. Baxter (57) reported that the ultraviolet spectrum for a similar structure, trans-1,2,4-trimethoxy-5-propenylbenzene (XXXV), exhibited two maxima at 253 and 302 mμ, and that the spectrum of the cis isomer was almost identical.



XXXV

The mass spectrum had a strong parent peak at m/e 238. An accurate mass determination of this ion confirmed the formula $C_{13}H_{18}O_4$. The base peak was observed at m/e 208 $(M-30)^+$, and the next most abundant peak was observed at m/e 193; which resulted by the loss of a methyl radical from the mass of 208. The corresponding metastable ion was observed at m/e 179. This is a fragmentation pattern very similar to component 19 which was tentatively identified as the corresponding cis isomer.

The infrared spectrum exhibited an absorption band at 955 cm^{-1}, characteristic of a trans arrangement of the -CH=CH- group. This band was absent from the spectrum of component 19. Two strong bands were observed at 1580 and 1500 cm^{-1} which are attributed to the skeletal vibrations of the substituted benzene ring. The absorption due to the C-H stretching of the one hydrogen atom of the aromatic ring was observed at 830 cm^{-1}. Absorption due to asymmetrical C-O-C stretching vibrations was observed at 1230 cm-1.

An authentic sample of trans-2,3,4,5-tetramethoxypropenyl-benzene was not available for confirmation of the identity of component 21.

Component 22

Component 22 was not identified. The parent peak of the mass spectrum was observed at m/e 222. The m/e 223 peak was measured as 16.66% of the parent peak. This percentage indicated 15 carbon atoms in the molecule and agrees with the proposed formula of $C_{15}H_{16}O$. A peak was observed at m/e 204 $(M-18)^+$ and a small peak was observed at m/e 205 $(M-17)^+$ which suggested that the compound was an alcohol. The infrared spectrum exhibited a band of medium intensity at 3450 cm^{-1} indicative of the O-H stretching of an alcohol.

Component 23

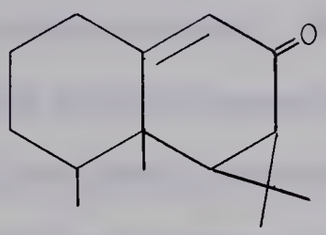
Component 23 was an unidentified compound which, by mass spectral evidence, had a molecular weight of 222 and a formula of $C_{15}H_{26}O$. The mass spectrum was very similar to the spectrum of component 22 (peaks at m/e 204 $(M-18)^+$ and m-e 205 $(M-17)^+$), which suggested that component 23 was also an alcohol. The suggestion is supported by a strong band observed at 3420 cm^{-1} in the infrared spectrum.

Component 24

Component 24 was not identified. It was present in the oil in only trace amounts and time permitted collection of only enough pure sample for an infrared spectrum. Strong bands were observed at 3400,2900,1710,1655,1450,1370, and 1265 cm^{-1}. Bands of medium intensity were observed at 1500,1412,1235,1130,1025,886 and 804 cm^{-1}.

Component 25

Aristolone (XXXVI) was isolated from the volatile oil of A. canadense, and identified by Bauer (19). It was the last component to emerge from the gas chromatographic column and constituted 0.1% of the oil. In the present investigation, component 25 was the last component to emerge from the Apiezon L column; therefore it was suspected that its identity could be aristolone.



XXX VI

The mass spectrum matched that of aristolone published by Bauer (19), with the exception that the parent peak m/e 218 was not the base peak. Major fragmentation peaks were observed at m/e 203, m/e 189, m/e 175, m/e 161, m/e 147, m/e 133, and m/e 119, which Bauer attributed to loss of CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, and C_7H_{15}, respectively, from the molecular ion; the charge remaining with the oxygen-containing fragment.

The infrared spectrum was consistent with the proposed structure in that a strong band was observed at 1660 cm^{-1}, a frequency characteristic of an α,β-unsaturated cyclic ketone. Absorption attributable to a gem-dimethyl group was observed at 1380 and 1360 cm^{-1}. Absorption due to $C-CH_3$ bending vibrations was observed at 1460 and 1380 cm^{-1}

The ultraviolet spectrum of component 25 was recorded and the maxima observed at 235, 310 and 333 mμ matched the published

(19,58) data, with the exception that the maximum at 333 mμ was not reported.

When this investigation was almost completed an F and M Model 700 gas chromatograph, equipped with dual Flame Ionization detectors and dual U.C. w98 columns, became available. The three samples of oil (A,B, and C) were chromatographed using this instrument (see experimental 4.4.1.2). Under optimum conditions 30 peaks were resolved in each sample of oil (see table 6), five more than previously resolved. The number of peaks resolved are attributed to the much increased sensitivity of this instrument over the instruments previously employed. It would have been desirable to use such conditions (column, instrument, sensitivity) throughout the course of this investigation, but this was not possible. The F and M 700 is limited to analytical separation, and no provisions exist for preparative separation. The number of constituents in Canadian snake-root oil was originally reported as 12, including a mixture of fatty acids (18). The number was extended to 13 in the work by Bauer (19). The present investigation has extended this number to 30 constituents (table 6), 25 of which have been isolated.

Four substances previously reported for this oil were not found in the present work. These compounds are geraniol, limonene, elemicin, and 3,4-dimethoxycinnamaldehyde.

An ethanolic extract of the powdered rhizome of A. canadense was prepared and separated into five fractions (neutral compounds; weak acids; strong acids; basic compounds; amphoteric compounds) using the fractionation system illustrated in figure 2.

The strong acid and basic extracts were chromatographed using TLC to determine if further investigation would be of interest.

The strong acid fraction, using acetonitrile: diethylamine: water (8:1:1) as solvent, was resolved into nine spots. The spot which had an R_F value of 0.62 was yellow and absorbed ultraviolet light. This is the behavior of aristolochic acid under these chromatographic conditions (31). Therefore it appears that there are a number of other acids present which could justify further investigation. Doskotch (31) is investigating the acids of A. canadense at this time.

The basic fraction was chromatographed using butanol: acetic acid: water (4:1:1) as solvent. The fraction was resolved into seven spots. One of the spots (R_F =0.42) had the same behavior as berberine under the same conditions. Both spots had the same R_F value, and fluoresced (orange) under ultraviolet irradiation. A spot with the same fluorescence was seen on the strong acid plate, which was expected as berberine is a quaternary base.

Berberine is a constituent of Hydrastis canadenses, a known contaminent of Aristolochia species (30). Therefore, if the sample of A. canadense used in this investigation was contaminated, it is possible that three of the bases found are berberine, hydrastine, and canadine, three constituents of H. canadenses.

III EXPERIMENTAL

3.1.0.0 Instruments, Apparatus, and Materials

The following instruments were used in this study:

1. F and M model 500 Gas Chromatograph, thermal conductivity detector.
2. F and M model 700 Gas Chromatograph, dual column, dual flame ionization detector.
3. Varian Aerograph model 712 Gas Chromatograph, flame ionization detector, automatic injection, automatic collection turntable.
4. Beckman IR 5A Infrared Spectrophotometer.
5. Beckman IR 10 Infrared Spectrophotometer.
6. Beckman DK2 Ultraviolet Spectrophotometer.
7. A.E.I. M.S.9 Mass Spectrophotometer.

The following gas chromatographic columns were employed in this study:

1. 10% Apiezon L on DMCS Chromosorb W, regular 60-80 mesh, 20 feet x 3/8 inch outside diameter (o.d.).
2. 10% Silicone Rubber SE 30 on Diatoport S, regular 60-80 mesh, 6 feet x 1/4 inch o.d.
3. 10% Apiezon L on Diatoport S, regular 60-80 mesh, 6 feet x 1/4 inch o.d.
4. 10% Diisodecyl Phthalate on Diatoport S, regular 60-80 mesh, 6 feet x 1/4 inch o.d.
5. 10% Polyphenylether 5 ring on Diatoport S, regular 60-80 mesh, 6 feet x 1/4 inch o.d.
6. 10% Lac-728 on Diatoport S, regular 60-80 mesh, 6 feet x 1/4 inch o.d.

7. 10% U.C. w98 on Diatoport S, regular 80-100 mesh, 6 feet x 1/8 inch o.d.

8. 2% Silicone Rubber SE 30 on acid washed DMCS Diatoport S, regular 60-80 mesh, 6 feet x 1/4 inch o.d.

The apparatus used in this study were:

1. Nester-Faust annular teflon spinning band distillation column.
2. Carl Ziess Abbe-refractometer.
3. Desaga S model SII T.L.C. spreader.
4. Buchler Flash evaporator.

The following materials were used in this study:

Asarum canadense root was supplied by Dominion Herb Co. Montreal. Three samples of the volatile oil of A. canadense were studied. Two were purchased commercially, one from the Dominion Herb. Co., the other from British Drug Houses, and hereafter will be referred to as oil A and oil B respectively. The third sample was freshly steam distilled from the powdered root and is designated oil C.

Authentic samples - The following terpenes were obtained commercially: α-pinene (K and K Laboratories), isoeugenol, β-pinene and methylisoeugenol (Aldrich Chemical Co. Inc.), geraniol, limonene, and linalool (Matheson Coleman and Bell), myrcene, α-terpineol, and β-terpineol (City Chemical Corp.), eugenol (Shawinigan), linalyl acetate (Eastman Organic Chemicals), bornyl acetate (J.T. Baker Chemical Co.). These were purified by preparative GC (Apiezon L column, F and M 500) and the required fraction collected.

1,2,3-Trimethoxybenzene was prepared from pyrogallol and dimethyl sulfate, using nitrogen instead of hydrogen (59), and

purified from hot aqueous ethanol, mp. 43-44°. (Lit. (59) mp. 47°.)

Methyleugenol was prepared in this department by E. Mah.

Thin Layer Chromatography (TLC) Plates

These were prepared by spreading a slurry of 30 g of Silica gel G (G. Merck and Co., Darmstadt) in 60 ml distilled water on 20x20 cm glass plates. The plates were coated to thickness of 0.25 mm and allowed to dry at room temperature for about one hour. They were then placed in an oven at 110° for thirty minutes and then stored in a dessicator of $CaSO_4$.

Spray reagents for TLC

Spray for acidic compounds: 0.3% solution of bromocresol green in water-methonol (20:80) containing 8 drops of 30% NaOH per 100 ml (60).

Spray for basic compounds: Potassium iodoplatinate reagent was prepared according to Bobbit (61).

All mass spectra are quoted in terms of relative abundance, with the most intense peak ("base peak") being taken as 100%.

All temperatures are in degrees centigrade.

Melting-points are uncorrected.

Chromatographic peak areas were calculated by triangulation.

3.2.0.0 Isolation of Steam-Volatile Oil C

3.2.1.0 One kilogram of dried milled rhizomes was extracted continuously with hot n-pentane (b p 35-36°) in a Soxhlet apparatus for one week and the solvent removed under reduced pressure. The residue (10.612 g, 1.06%) was steam distilled (5 hrs) and the distillate

extracted with ether. Evaporation of the solvent yielded 1.66 g (0.166%) of a fragrant light-yellow oil (C).

3.2.2.0 Dried milled rhizomes (500 g) were steam distilled (15 hrs) and the distillate extracted with ether. Evaporation of the solvent yielded 6.233 g (1:24%) of a fragrant light-yellow oil (C).

3.3.0.0 Attempted Separation of the Constituents of Oil of Canadian Snake-root by Fractional Distillation

3.3.1.0 A portion of the oil (25 ml) (sample A) was fractionated under reduced pressure (3 mm Hg) in a Nester Faust "spinning band" distillation assembly. The collection scheme for the oil is shown in Table 2.

Table 2

Distillation of Oil of Canadian Snake-root

Distillation Cuts	Volume
65° to 67°	0.6 ml
68° to 70°	0.2
70° to 72°	0.1
72°	1.5
72° to 74°	0.7
74° to 76°	0.3
76° to 78°	0.2
78° to 80°	0.2
80° to 82°	0.6
82°	4.0
100° to 102°	0.2
104° to 106°	0.2
108° to 110°	0.1
112° to 114.5°	4.0

115° to 118°	2.9
119.5° to 122°	0.7
122°	1.5
120°	0.3

Each of the fractions shown in Table 2 were chromatographed on the 6 foot Apiezon L column. The resulting chromatograms indicated a great deal of overlapping between fractions.

3.3.2.0 A portion of the oil (25 ml) was fractionated under reduced pressure (2 mm Hg) using a fractionating column packed with glass beads. Fractions were collected as follows:

Fraction	Distillation range
A	60 to 66°
B	68 to 74°
C	75 to 93°
D	> 94°

Some fragrant pale-yellow oil condensed in the vacuum pump trap and was collected and labelled Fraction E.

3.3.2.1 The five fractions obtained as described in section 3.3.2.0 were chromatographed on the SE 30 column. The carrier gas flow was maintained at 40 ml/min for all runs. The results are shown in Table 3.

Table 3

Composition of Fractions A-E Obtained by Distillation

Fraction	Column temp.*	No. of peaks	Retention times (mins)
E	90-275°	9	6.53, 6.70, 7.54, 9.05, 10.04, 10.78, 12.36, 13.76, 15.56.
A	90.275°	11	7.42, 8.14, 10.04, 11.10,

			12.16, 12.42, 13.98, 15.84, 16.72, 18.26, 18.78.
B	90-275°	13	7.70, 8.98, 9.02, 11.38, 12.44, 12.80, 14.50, 16.42, 17.36, 18.96, 19.44, 20.50, 21.84.
C	90-275°	15	7.36, 9.18, 10.78, 12.50, 14.16, 16.08, 17.02, 18.80, 19.42, 20.00, 20.56, 21.82, 22.40, 23.40, 25.16.
D	90-275°	12	18.40, 19.82, 20.88, 22.20, 23.44, 24.56, 25.12, 26.00, 26.62, 27.00, 28.90, 29.66.

* Temperature increased at 5.6°/min

3.3.2.2 An infrared spectrum of a thin film of each fraction collected as described in section 3.3.2.0 was recorded on a Beckman IR5A spectrophotometer. Major absorption bands are listed:

Fraction E: 3450 (s), 3360 (s), 3050 (s), 2950 (s), 1740 (sh), 1710 (s), 1635 (s), 1590 (m), 1440 (s), 1380 (s), 1250 (m), 1170 (m), 1110 (m), 1050 (m), 1015 (m), 990 (s), 885 (s), 815 (s), 785 (s) cm^{-1}.

Fraction A: 3450 (s), 3050 (m), 2940 (s), 2900 (s), 1725 (s), 1635 (m), 1445 (s), 1405 (m), 1365 (s), 1255 (sh), 1240 (s), 1175 (m), 1110 (m), 1020 (s), 995 (m), 920 (s), 835 (s), 800 (m), 740 (m), 690 (m) cm^{-1}.

Fraction B: 3500 (m), 2945 (s), 2900 (s), 1725 (s), 1450 (m), 1370 (s), 1250 (s), 1165 (m), 1110 (m), 1020 (m), 920 (m), 840 (m), 800 (m) cm^{-1}.

Fraction C : 3500 (s), 3450 (s), 3070 (m), 2925 (s), 2900 (s), 1725 (s), 1635 (m), 1590 (m), 1505 (s), 1450 (s), 1370 (s), 1260 (s), 1240 (s), 1035 (s), 920 (s), 835 (m), 805 (s), 765 (m), 745 (m), 690 (m) cm^{-1}.

Fraction D: 3500 (s), 3050 (m), 2925 (s), 1725 (s), 1640 (s), 1585 (s), 1505 (s), 1450 (s), 1335 (m), 1260 (s), 1235 (s), 1135 (s), 1030 (s), 965 (m), 915 (s), 850 (s), 810 (s), 780 (m), 765 (s) cm^{-1}.

3.4.0.0 Gas Liquid Chromatography

3.4.1.0 Analysis of Oil of Canadian Snake-root (Sample A)

A portion of the oil (3 µl) was injected into the 6 foot SE 30 column installed in an F and M model 500 gas chromatograph. Conditions for optimum resolution were:

Column Temperature: Step-wise linear programmed 105° for 16 min, 125° for 7 min, 150° for 8 min, 200° for 7 min, 225° for 7 min, 250° held.

Injection Temperature: 260°

Detection Temperature: 275°

Carrier Gas: Helium

Carrier Gas Flow Rate: 60 ml/min

Bridge Current: 170 mA

Attenuation: x2

Using the above conditions, the oil was resolved into nineteen components. See Table 4.

Table 4

Retention Times of Components of Oil A

Peak No.	Retention time (mins)
1	4.40
2	4.80
3	5.40
4	7.10
5	9.06
6	10.40
7	14.00
8	18.26
9	22.70
10	24.70
11	28.40
12	31.40
13	33.44
14	34.80
15	36.36
16	38.00
17	39.46
18	40.46
19	43.00

3.4.1.1 Another sample (20 µl) of the same oil was injected into the 6 foot Apiezon L column under the following conditions:

Initial column temperature: 100°

Final column temperature: 250° held

Program rate: 4°/min

Injection temperature: 260°

Detector temperature: 275°

Carrier gas: Helium

Carrier gas flow: 70 ml/min

Bridge current: 170 mA

Attenuation: x32

The above conditions resulted in the oil being resolved into twenty-five components. See Table 5.

Table 5

Retention Times (in mins) of Components of Oil of Canadian Snake-root (Sample A)

PEAK	TIME	PEAK	TIME	PEAK	TIME
1	7.20	10	16.56	19	24.68
2	8.02	11	17.60	20	25.46
3	8.80	12	18.28	21	26.24
4	10.18	13	18.50	22	27.46
5	11.00	14	20.64	23	28.16
6	12.60	15	21.56	24	29.20
7	13.40	16	21.80	25	30.40
8	14.94	17	23.38		
9	15.60	18	24.00		

3.4.1.2 The three samples (0.5 µl) of oil of Canadian snake-root (Sample A,B, and C) were chromatographed on the 6 foot U.C. w98 column installed in the F and M model 700 gas chromatograph. Conditions employed were:

Initial Column Temperature: 50°

Final Column Temperature: 170° held

Program rate: 5°/min

Carrier Gas: Nitrogen

Carrier Gas Flow Rate: 50 ml/min

Range 100

Attenuation: x20

The resulting chromatograms were qualitatively similar, but they differed quantitatively. See Table 6.

Table 6

Composition of Samples A,B, and C of Oil of Canadian Snake-root

Peak	Peak areas (%) in Sample A	B	C
i	1.89	2.41	0.01
ii	0.94	1.19	trace
iii	3.06	2.25	0.06
iv	0.79	0.95	0.02
v	1.22	1.50	trace
vi	0.16	trace	0.07
vii	0.35	0.40	0.12
viii	7.82	9.72	0.23
ix	1.20	0.32	0.03
x	0.48	0.73	trace
xi	6.62	4.66	0.16
xii	0.15	0.57	0.02
xiii	22.25	20.89	0.72
xiv	1.94	1.42	0.10
xv	trace	0.27	trace
xvi	4.81	0.49	trace
xvii		0.48	trace

Table 6 (Continued)

xviii	24.60	29.64	90.26
xix	3.20	0.86	trace
xx	1.14	0.97	0.41
xxi	6.89	trace	0.22
xxii	0.58	0.77	0.14
xxiii	1.64	1.13	3.70
xxiv	0.96	4.46	0.70
xxv	2.57	2.05	1.10
xxvi	0.52	1.50	1.28
xxvii	0.27	0.38	0.13
xxviii	trace	0.17	0.32
xxix	3.94	9.83	0.13
xxx	trace	trace	0.10

3.4.2.0 Separation and Collection of Components of Oil of Canadian Snake-root (Sample A).

3.4.2.1 Preliminary Separation

Initial separations were performed on a Varian 712 gas chromatograph. A sample (25-50 µl), to which toluene was added, was automatically injected into the 20 foot Apiezon L column. The conditions found most suitable are described below:

Initial column temperature: 120°

Final column temperature: 260° held

Program rate: 10°/min

Injection temperature: 295°

Detection temperature: 295°

Carrier gas: Nitrogen

Carrier gas flow: 150 ml/min

Range: 1

Attenuation: x64

The eluate was collected arbitrarily in five fractions (Fraction I - V), using 10 ml collection bottles. The upper chamber of each bottle was plugged with steel wool, and the bottles were cooled in a dry ice-isopropyl alcohol mixture during collection. A recorder trip switch was used to actuate the collection turntable. When this was set at 80% the five fractions shown in Figure 1 were obtained. The solvent peak, and peaks 8 and 14 tripped the turntable actuating switch.

3.4.2.2. Final Separation and Collection

The five fractions collected as described in section 3.4.2.1 were rechromatographed on the 6 foot Apiezon L column installed in the F and M 500. Samples of each fraction (20 µl) were injected on to the column and the column temperatures were maintained constant. The temperatures found most suitable for each fraction were: Fraction 1, 110°; Fraction II, 160°; Fraction III, 175°; Fraction IV, 180°; Fraction V, 200°. Carrier gas flow rate was maintained at 100 ml/min for all runs.

The pure components emerging from the column were collected in unsealed melting point capillary tubes inserted through a hole in a rubber septum installed at the exit tip. The collection tubes were cooled by means of a wick soaked in methylene chloride wrapped around the tube. Compounds 6-25 did not require cooling, and were collected at room temperature. The composition of each fraction is illustrated in Figure I.

Purity of components collected from GC, before submission for mass spectral analysis, was checked by thin layer chromatography.

The separation medium used was alumina (M. Woelm). The alumina was made into a slurry with chloroform. Microscopic slides were dipped into this slurry and allowed to air dry. Development of the coated plate, without activation, was carried out in a small unsaturated chamber. The eluting solvent used was hexane. The developed plate was first visualized under ultraviolet irradiation. Following this, the plates were sprayed with 1% vanillin in concentrated sulfuric acid (62).

3.4.3.0 Gas Chromatography of Fraction 5 Using Internal Standard

Fraction 5 was chromatographed on the 6 foot Apiezon L column at 170°. For purposes of defining emergence time independent of day to day fluctuations of temperature and gas flow rate, an internal standard (1,2,3-trimethoxybenzene) was incorporated directly in the fraction. Table 7 presents the relative retention times of fraction 5.

Table 7

Relative Retention Times of Components of Canadian Snake-root

Peak	
14	1.57*
15	2.05
16	2.47
17	2.74
18	3.00
19	3.55
20	4.02
21	4.93
22	5.60

23	6.32
24	-
25	-

* Retention times are relative to that of 1,2,3-trimethoxybenzene which is given the value of 1.

3.4.4.0 Gas Chromatography of Methylisoeugenol

Methylisoeugenol (1 µl) was injected into the 2% SE 30 column installed in the F and M 500 gas chromatograph. Conditions for optimum resolution were:

Column Temperature:	180°
Injection Temperature:	260°
Detection Temperature:	265°
Carrier Gas:	Helium
Carrier Gas Flow Rate:	75 ml/min
Bridge Current:	150 mA
Attenuation:	x8

Using the above conditions two peaks were resolved corresponding to cis-methylisoeugenol, and trans-methylisoeugenol. Retention times for these compounds were: 4.0, and 5.0 minutes. The relative percentages of the sample were: cis-methylisoeugenol 4.0%, trans-methylisoeugenol 96.0%.

3.5.0.0 Properties of Components of Oil of Canadian Snake-root

Mass spectra were recorded on an A.E.I. M.S. 9 mass spectrophotometer with the heated inlet temperature set at 185°. Infrared spectra were recorded on either a Beckman IR 5A or IR 10 spectrophotometer as thin films. Refractive indices were determined at 20° on a Carl Ziess refractometer. Ultraviolet spectra were recorded on a Beckman DK 2 ultraviolet spectrophotometer using 95%

ethanol as solvent.

Component 1

Colorless oil.

Infrared spectrum: 2905 (s), 1087 (w) cm^{-1}.

Mass spectrum:

m/e	137	136	121	107	105	94	93	92	91	81	80
%	1.85	13.0	13.7	6.0	6.3	10.7	100	29.0	18.5	5.6	9.3
	79	77	68	67	55	53	44	43	41	39	32
	14.5	18.0	7.8	7.4	7.0	7.4	31	10.0	15.5	10.0	43.0

An accurate mass determination of the molecular ion m/e 136 was made: measured: 136.1252; calculated for $C_{10}H_{16}$: 136.1252.

Component 2

Colorless oil.

Infrared spectrum: 3020 (m), 2920 (s), 2855 (s), 2800 (sh), 1587 (m), 1428 (m), 1368 (m), 986 (m), 903 (sh), 889 (s) cm^{-1}.

An infrared spectrum of an authentic sample of myrcene was also recorded: 3060 (m), 2940 (s), 2855 (s), 1580 (m), 1430 (m), 1374 (m), 991 (m), 906 (sh), 890 (s) cm-1.

Mass spectrum:

m/e	137	136	121	107	95	94	93	92	91	80	79
%	1.25	9.2	10.6	5.3	5.1	11.3	100	9.2	10.4	7.7	15.8
	77	70	69	68	67	55	53	43	41	39	
	11.5	5.7	85.0	9.0	13.3	6.4	10.3	7.8	87.5	16.7	

Component 3

Colorless oil.

Infrared spectrum: 3040 (w), 2942 (sh), 2900 (s), 1635 (m), 1450 (m), 1439 (m), 1380 (m), 1365 (m), 875 (s), 853 (w) cm^{-1}.

An infrared spectrum of an authentic sample of β-pinene was also recorded: 3040 (w), 2941 (sh), 2900 (s), 1638 (m), 1460 (m), 1433 (m), 1379 (m), 1364 (m), 874 (s), 853 (m) cm^{-1}.

Mass spectrum:

m/e	137	136	121	94	93	92	91	80	79	77	69
%	1.15	11.3	9.5	12.3	100	7.9	10.4	10.0	15.5	12.8	40.0
	67	55	53	43	41	39					
	7.0	5.9	6.7	5.0	38.5	10.0					

An accurate mass determination of the molecular ion m/e 136 was made: measured: 136.1252; calculated for $C_{10}H_{16}$: 136.1252.

Component 4

Colorless oil.

Infrared spectrum: 2925 (s), 2875 (sh), 2828 (sh), 1430 (m), 1370-1354 (m), 1205 (w), 1050 (m), 985 (m), 885 (m), 813 (s) cm^{-1}.

Mass spectrum:

m/e	135	134	120	119	117	115	111	108	97	96	95	94
%	4.3	30.2	10.2	100	10.2	5.9	19.2	8.8	6.2	5.5	7.1	21.0
	93	91	84	83	81	79	77	71	69	68	67	65
	24.7	22.5	8.6	7.4	19.8	11.6	10.8	18.4	13.0	25.4	18.0	7.7
	59	58	55	53	51	44	43	41	39	31	30	
	45.0	9.6	16.3	10.2	6.5	9.6	98.0	30.4	21.2	7.7	5.0	

An accurate mass determination of the molecular ion m/e 134 was made: measured 134.1096; calculated for $C_{10}H_{14}$: 134.1097.

Component 5

Colorless oil.

Refractive index: $n_D^{20} = 1.4605$

Refractive index of linalool: $n_D^{20} = 1.4604$ (39)

Infrared spectrum: 3340 (s), 2925 (s), 2870 (s), 2820 (sh), 1430 (s), 1400 (m), 1363 (m), 1105 (s), 915 (s), 827 (m) cm^{-1}.

An infrared spectrum of an authentic sample of linalool was also recorded: 3340 (s), 2925 (s), 2870 (s), 2820 (sh), 1430 (s), 1400 (m), 1363 (m), 1105 (s), 915 (s), 827 (m) cm^{-1}.

Component 6

Colorless oil.

Infrared spectrum: 3330 (s), 2890 (s), 2820 (sh), 1450-1429 (m), 1369-1349 (m), 1123 (m), 1098 (m), 914 (m), 907 (m), 882 (m) cm^{-1}.

Mass spectrum:

m/e	155	154	139	137	136	125	121	111	107	97	96
%	0.67	6.0	8.7	5.7	36.0	8.0	27.0	17.0	17.5	12.6	32.0
	95	94	93	92	91	84	83	82	81	80	79
	11.0	5.7	30.0	7.0	8.0	8.8	10.5	14.6	100	24.0	13.0
	77	72	71	70	69	68	67	59	58	57	56
	8.0	7.0	18.0	5.3	29.0	9.0	14.0	8.5	30.0	5.3	5.2
	55	54	53	44	43	41	39	32			
	29.5	5.7	10.6	10.0	51.5	30.0	14.0	22.8			

An accurate mass determination of the molecular ion m/e 154 was made: measured 154.1359; calculated for $C_{10}H_{18}O$: 154.1358.

Component 7

Colorless oil.

Infrared spectrum: 3380 (s), 2900 (s), 2820 (sh), 1636 (w), 1442 (m), 1370 (m), 1220 (m), 1148 (m), 1120 (m), 1000 (m), 948 (m), 910 (s), 885 (s) cm^{-1}.

Mass spectrum:

m/e	153	152	139	137	136	121	111	109	108	107	97
%	1.11	10.2	7.6	7.6	50.7	30.0	12.0	9.3	25.0	29.3	7.6
	96	95	94	93	92	91	85	84	83	82	81
	12.0	33.0	29.5	52.5	22.0	13.3	5.8	23.5	22.0	12.9	51.0
	80	79	77	72	71	70	69	68	67	59	58
	51.0	19.5	8.0	8.0	100	13.3	48.0	47.0	26.0	5.8	29.0
	56	55	54	53	44	43	42	41	40	39	32
	9.3	34.0	5.0	18.6	16.0	63.0	6.7	48.0	6.2	22.0	26.0

An accurate mass determination of the molecular ion m/e 152 was made: measured: 152.1202; calculated for $C_{10}H_{16}O$: 152.1201.

Component 8

Colorless to pale-yellow oil.

Infrared spectrum: 2985 (m), 2920 (m), 2860 (w), 1740 (s), 1645 (w), 1445 (m), 1410 (m), 1365 (s), 1240 (s), 1165 (w), 1105 (w), 1085 (w), 1010 (m), 915 (m), 820 (w) cm^{-1}.

An infrared spectrum of an authentic sample of linalyl acetate was also recorded: 2985 (m), 2920 (m), 2858 (w), 1740 (s), 1645 (w), 1445 (m), 1410 (m), 1365 (s), 1240 (s), 1165 (w), 1105 (w), 1085 (w), 1010 (m), 912 (m), 820 (w) cm^{-1}.

Mass spectrum:

m/e	137	136	121	107	105	95	94	93	92	91	81
%	2.5	21.4	22.2	9.2	8.6	8.0	13.0	100	17.5	25.8	8.3
	80	79	77	69	68	67	65	60	55	53	51
	14.5	25.0	20.0	34.0	22.0	19.0	6.4	9.2	8.5	13.9	5.6
	45	43	41	39							
	11.4	21.0	50.0	20.0							

An accurate mass determination of the ion m/e 136 was made:

measured: 136.1252; calculated for $C_{10}H_{16}$: 136.1252.

Component 9

Colorless oil.

Infrared spectrum: 3340 (s), 2938 (s), 2895 (s), 2820 (sh), 1436 (m), 1370 (s), 1282 (w), 1220 (m), 1156 (s), 1130 (s), 1108 (sh), 1043 (w), 1020 (w), 947 (w), 923-914 (s), 834 (m), 797 (m) cm^{-1}.

An infrared spectrum of an authentic sample of α-terpineol was also recorded: 3340 (s), 2940 (s), 2900 (s), 2820 (sh), 1438 (m), 1370 (s), 1282 (w), 1220 (m), 1155 (s), 1128 (s), 1108 (sh), 1041 (w), 1020 (w), 948 (w), 924-912 (s), 834 (m), 797 (m) cm^{-1}.

Mass spectrum:

m/e	139	137	136	121	107	96	95	94	93	92	91
%	8.1	6.2	53.0	53.0	6.6	7.0	15.5	9.8	61.0	19.0	9.4
	81	80	79	77	71	69	68	67	59	55	53
	39.0	6.0	14.5	10.0	9.2	8.6	15.5	17.4	100	12.6	8.6
	43	41	39	31							
	34.0	21.0	11.4	7.8							

An accurate mass determination of the ion m/e 136 was made:

measured: 136.1252; calculated for $C_{10}H_{16}$: 136.1252.

Component 10

Colorless oil.

Refractive index: $n_D^{20} = 1.4622$

Refractive index of bornyl acetate: $n_D^{20} = 1.4623$ (39).

Infrared spectrum: 2920 (s), 2860 (sh), 1725 (s), 1470 (sh), 1448 (m), 1375 (s), 1361 (s), 1300 (m), 1243 (s), 1162 (w), 1138 (w), 1112 (m), 1048 (m), 1034 (s), 995 (w) cm^{-1}.

An infrared spectrum of an authentic sample of bornyl acetate was also recorded: 2925 (s), 2860 (sh), 1730 (s), 1472 (sh), 1448 (m), 1377 (s), 1361 (s), 1300 (m), 1246 (s), 1162 (w), 1139 (w), 1112 (m), 1048 (m), 1033 (s), 995 (w) cm^{-1}.

Mass spectrum:

m/e	197	196	154	137	136	122	121	111	110	109	108	
%	0.60	4.6	8.6	6.6	51.5	5.3	57.6	5.3	11.9	13.9	17.9	
	107	105	96	95	94	93	92	91	83	82	81	
	13.9	6.6	8.6	100	13.9	68.5	18.5	15.9	6.0	9.3	13.9	
	80	79	77	71	69	68	67	55	53	43	41	39
	18.5	18.5	13.0	7.3	20.0	14.5	19.0	20.0	11.9	85.5	43.0	17.9

An accurate mass determination of the molecular ion m/e 196 was made: measured: 196.1457; calculated for $C_{12}H_{20}O_2$: 196.1463.

Component 11

Colorless oil.

Refractive index: $n_D^{20} = 1.4569$

Infrared spectrum: 2945 (s), 2900 (s), 2850 (sh), 1730 (s), 1440 (m), 1378 (m), 1360 (m), 1232 (s), 1122 (m), 955 (w) cm^{-1}.

Mass spectrum:

m/e	136	123	121	95	94	93	91	82	81	80	79
%	5.7	6.1	7.7	10.4	5.0	21.0	12.6	9.4	19.5	8.0	16.6
	77	70	69	68	67	56	55	53	52	51	45
	11.0	5.0	32.5	28.6	26.6	5.8	17.7	22.0	5.0	8.2	10.4
	43	42	41	40	39	32					
	82.0	9.0	100	10.0	44.0	7.3					

An accurate mass determination of the ion m/e 136 was made: measured: 136.1252; calculated for $C_{10}H_{16}$: 136.1253.

Component 12

Colorless oil.

Refractive index: $n_D^{20} = 1.4632$

Infrared spectrum: 2945 (s), 2900 (s), 2850 (sh), 1725 (s), 1440 (m), 1380 (s), 1360 (s), 1260-1230 (s), 1180 (w), 1155 (m), 1132 (m), 1022 (m), 955 (w) cm^{-1}.

Mass spectrum:

m/e	137	136	121	107	105	94	93	92	91	81	80
%	2.6	23.5	26.5	9.1	9.8	14.4	100	20.0	31.0	6.8	25.7
	78	69	68	67	65	60	55	53	51	45	43
	24.0	32.5	24.0	18.0	7.6	8.3	8.3	12.9	6.8	9.1	14.5
	41	39	32								
	46.0	20.5	32.0								

An accurate mass determination of the ion m/e 136 was made:

measured: 136.1252; calculated for $C_{10}H_{16}$: 136.1252.

Component 13

Pale-yellow oil.

Infrared spectrum: 3500 (s), 3030 (w), 2940 (m), 2820 (w), 1635 (m), 1610 (m), 1510 (s), 1461-1450 (s), 1430 (m), 1370 (m), 1266 (s), 1235 (s), 1205 (m), 1150 (s), 1123 (s), 1035 (s), 998 (m), 920 (s), 855 (m), 818 (s), 795 (s), 747 (s) cm^{-1}.

An infrared spectrum of an authentic sample of eugenol was also recorded: 3500 (s), 3030 (w), 2940 (m), 2820 (w), 1635 (m), 1610 (m), 1510 (s), 1461-1450 (s), 1429 (m), 1363 (m), 1266 (s), 1235 (s), 1205 (m), 1150 (m), 1120 (s), 1035 (s), 998 (m), 920 (s), 855 (m), 818 (s), 795 (s), 747 (s) cm^{-1}.

Mass spectrum:

m/e	165	164	149	137	133	132	131	123	122	121	111
%	10.0	100	38.6	21.6	21.0	11.6	33.0	5.7	5.7	24.4	8.2
	109	107	105	104	103	97	95	94	93	92	91
	6.7	6.4	11.8	27.3	35.2	9.0	9.9	11.2	15.4	5.2	34.0
	83	81	79	78	77	71	69	68	67	66	65
	10.4	12.7	16.7	15.0	46.0	8.5	15.9	5.5	12.7	7.3	17.6
	63	60	57	55	53	52	51	50	45	44	43
	10.7	5.7	19.3	36.4	10.1	9.7	24.8	9.7	9.7	6.6	26.1
	41	39	32								
	28.4	30.7	12.7								

An accurate mass determination of the molecular ion m/e 164 was made: measured: 164.0837; calculated for $C_{10}H_{12}O_2$: 164.0837.

Component 14

Pale-yellow oil.

Relative retention time: 1.57

Relative retention time of methyleugenol: 1.57 (52).

Ultraviolet spectrum: λmax. 228, 280 mμ.

Infrared spectrum: 3080 (w), 3000 (m), 2940 (s), 2900 (m), 2840 (m), 1640 (s), 1610 (s), 1594 (s), 1515 (s), 1465 (s), 1418 (s), 1336 (m), 1260 (s), 1234 (s), 1188 (m), 1153 (s), 1140 (s), 1028 (s), 990 (m), 945 (w), 908 (s), 840 (s), 800 (s), 760 (s), 740 (s) cm^{-1}.

An infrared spectrum of an authentic sample of methyleugenol was also recorded: 3080 (w), 3000 (m), 2940 (s), 2900 (m), 2840 (m), 1640 (s), 1610 (s), 1594 (s), 1515 (s), 1465 (s), 1418 (s), 1336 (m), 1260 (s), 1234 (s), 1188 (m), 1153 (s), 1140 (s), 1028 (s), 990 (m), 945 (w), 908 (s), 840 (s), 800 (s), 760 (s), 740 (s) cm^{-1}.

Mass spectrum:

m/e	179	178	163	151	147	135	115	107	105	103	91
%	10.7	100	26.4	9.0	23.0	6.6	6.3	13.5	6.3	19.0	18.4
	79	77	65	51	41	39					
	7.5	10.4	7.2	7.5	7.8	6.9					

An accurate mass determination of the molecular ion m/e 178 was made: measured: 178 0995; calculated for $C_{11}H_{14}O_2$: 178.0994.

Component 15

Pale-yellow oil.

Relative retention time: 2.05

Relative retention time of cis-methylisoeugenol: 2.08 (52).

Ultraviolet spectrum: λmax. 255 mμ.

An ultraviolet spectrum of an authentic sample of cis-methylisoeugenol was recorded: λmax. 255 mμ.

Infrared spectrum: 2995 (m), 2920 (s), 2820 (m), 1600 (s), 1575 (s), 1508 (s), 1450 (s), 1410 (m), 1365 (m), 1335 (m), 1250 (s), 1235 (s), 1140 (s), 1030 (s), 860 (s), 810 (s), 765 (s), 710 (w) cm^{-1}.

An infrared spectrum of an authentic sample of cis-methylisoeugenol was also recorded: 2995 (m), 2920 (s), 2817 (m), 1595 (s), 1572 (s), 1502 (s), 1450 (s), 1412 (m), 1360 (m), 1335 (m), 1250 (s), 1235 (s), 1138 (s), 1025 (s), 860 (s), 810 (s), 765 (s), 710 (w) cm^{-1}.

Mass spectrum:

m/e	179	178	163	147	135	115	107	105	103	91	79
%	12.6	100	34.5	10.0	6.7	9.2	22.7	10.0	15.0	22.0	10.0
	77	65	55	51	41	39	32				
	12.6	8.5	5.0	7.0	10.0	10.0	21.0				

An accurate mass determination of the molecular ion m/e 178 was made: measured: 178.0995; calculated for $C_{11}H_{14}O_2$: 178.0994.

Component 16

Pale-yellow oil.

Infrared spectrum: 3080 (w), 3000 (sh), 2960 (s), 2930 (s), 2840 (m), 1730 (sh), 1682 (s), 1590 (s), 1510 (s), 1460 (s), 1422 (s), 1395 (m), 1340 (m), 1265 (s), 1240 (s), 1150 (s), 1133 (s), 1018 (s), 860 (m), 800 (s), 775 (m), 722 (s) cm^{-1}.

Mass spectrum:

m/e	219	218	204	203	202	191	189	187	185	176	175
%	5.6	27.5	8.6	33.0	15.0	7.0	9.8	10.7	7.0	6.2	21.0
	173	167	166	165	164	163	162	161	160	159	151
	6.5	7.0	58.0	42.0	44.0	15.0	9.3	23.0	8.8	26.0	21.6
	150	149	148	147	146	145	143	139	137	136	135
	7.2	21.0	8.6	31.0	8.1	21.0	7.0	5.8	20.0	7.9	13.0
	134	133	132	131	129	128	126	125	124	123	122
	5.6	32.0	11.0	27.0	10.0	10.7	7.0	6.3	30.0	17.5	14.0
	121	120	119	118	117	115	111	110	109	108	107
	23.0	10.0	37.0	7.0	20.0	15.0	9.3	11.5	28.0	13.7	25.0
	106	105	104	103	97	96	95	94	92	91	84
	11.7	51.0	8.9	13.0	13.0	12.5	37.0	9.5	11.5	58.0	5.3
	83	82	81	80	79	78	77	76	71	69	68
	9.0	9.5	26.0	10.0	40.0	13.0	51.0	6.3	11.5	19.0	6.5
	67	66	65	64	63	60	57	56	55	53	52
	34.0	5.5	28.0	5.5	14.0	6.5	7.0	6.3	40.0	22.0	10.9
	51	50	45	44	43	42	41	40	39	38	32
	28.0	9.8	10.2	56.0	100	8.4	86.0	8.4	42.0	5.1	34.2
	31										
	5.4										

An accurate mass determination of the molecular ion m/e 218 was made: measured: 218.1667; calculated for $C_{15}H_{22}O$: 218.1670.

Component 17

Pale-yellow oil.

Relative retention time: 2.74

Relative retention time of trans-methylisoeugenol: 2.74 (52).

Ultraviolet spectrum: λ max. 258 mμ.

An ultraviolet spectrum of an authentic sample of trans-methylisoeugenol was recorded: λ max. 258 mμ.

Infrared spectrum: 2920 (sh), 2860 (s), 2760 (m), 1585 (m), 1562 (m), 1490 (s), 1440 (s), 1400 (m), 1318 (m), 1285 (m), 1250 (s), 1222 (s), 1150 (s), 1130 (s), 1020 (s), 960 (s), 852 (s), 810 (s), 780 (s) cm^{-1}.

An infrared spectrum of an authentic sample of trans-methylisoeugenol was also recorded: 2920 (sh), 2860 (s), 2760 (m), 1585 (m), 1562 (m), 1490 (s), 1440 (s), 1400 (m), 1318 (m), 1285 (m), 1250 (s), 1222 (s), 1150 (s), 1130 (s), 1020 (s), 960 (s), 852 (s), 810 (s), 780 (s) cm^{-1}.

Mass spectrum:

m/e	179	178	163	147	145	135	133	132	131	120	119
%	11.8	100	34.5	8.4	5.6	6.0	6.3	14.5	6.7	6.5	19.0
	117	115	107	105	103	91	79	77	65	51	41
	6.0	7.6	18.4	14.5	13.0	21.0	8.9	10.9	7.0	6.0	11.8
	39										
	7.0										

Component 18

Pale-yellow oil.

Relative retention time: 3.00

Relative retention time of 2,3,4,5-tetramethoxyallylbenzene: 3.01 (52).

Ultraviolet spectrum: λ max. 228,282 mμ.

Mass spectrum:

m/e	239	238	208	204	194	193	192	189	165	161	152
%	1.11	9.1	19.0	11.0	21.0	11.0	8.6	6.3	6.7	13.5	10.7
	151	147	136	135	134	133	123	121	120	119	109
	100	5.2	5.0	8.0	5.8	8.6	5.2	9.7	5.7	23.0	14.6
	108	107	106	105	95	94	93	92	91	81	80
	9.4	20.0	7.3	17.6	10.7	12.8	41.0	7.6	24.0	15.0	7.7
	79	78	77	71	69	68	67	65	55	53	51
	21.0	7.4	17.0	7.7	50.0	6.4	16.7	11.7	15.7	11.4	7.4
	43	41	39								
	27.0	49.0	17.6								

An accurate mass determination of the molecular ion m/e 238 was made: measured:238.1211; calculated for $C_{13}H_{18}O_4$: 238.1205.

Component 19

Yellow oil.

Relative retention time: 3.55

Relative retention time of cis-2,3,4,5-tetramethoxypropenylbenzene: 3.52 (52).

Ultraviolet spectrum: λmax. 258,282 mμ.

Infrared spectrum: 2920 (s), 2840 (sh), 1640 (m), 1585 (s), 1495 (s), 1460 (s), 1370 (m), 1330 (m), 1235 (s), 1130 (s), 1010 (m), 885 (s) cm^{-1}.

Mass spectrum:

m/e	239	238	224	209	208	205	204	196	194	193	192
%	2.1	15.0	6.0	6.8	43.5	8.2	42.0	8.6	7.7	38.5	8.9
	190	189	176	175	165	163	162	161	159	157	151
	5.1	30.0	48.0	10.0	12.7	7.7	13.6	57.0	8.4	6.3	17.0
	150	149	148	147	145	137	136	135	134	133	132
	5.5	9.6	15.5	30.0	7.7	6.8	10.4	27.0	19.5	38.0	5.8

m/e	131	123	122	121	120	119	118	117	115	109	108
%	10.4	13.0	17.0	37.0	19.0	50.0	5.0	9.0	7.7	35.0	34.0
	107	106	105	104	103	96	95	94	93	92	91
	58.0	20.0	71.0	5.0	7.7	5.2	32.0	29.0	95.5	23.5	61.0
	83	82	81	80	79	78	77	71	70	69	68
	6.4	14.0	50.0	19.5	55.5	13.6	44.0	7.3	5.5	67.0	13.6
	67	66	65	63	59	57	56	55	53	52	51
	39.0	7.7	23.0	6.8	13.6	13.0	5.5	52.0	31.5	6.4	13.6
	43	42	41	40	39						
	36.0	7.7	100	8.0	40.0						

An accurate mass determination of the molecular ion m/e 238 was made: measured: 238.1211; calculated for $C_{13}H_{18}O_4$: 238.1205.

Component 20

Yellow viscous oil.

Infrared spectrum: 2920 (s), 2860 (m), 1730-1630 (m), 1590 (m), 1510 (m), 1455 (s), 1375 (s), 1365 (m), 1325 (w), 1255 (m), 1140 (s), 1110 (s), 1080 (s), 1020 (s), 985 (m), 880 (s) cm^{-1}.

Mass spectrum:

m/e	223	222	208	207	206	205	204	202	200	190	189
%	2.5	19.0	15.5	100	5.0	5.2	26.4	10.4	10.5	5.0	28.0
	179	175	165	164	163	162	161	160	159	158	157
	6.4	6.3	6.2	23.6	8.5	13.5	67.0	7.2	31.0	5.0	30.0
	151	150	149	148	147	145	143	142	141	138	137
	18.0	7.2	27.0	52.0	13.0	9.0	6.2	9.6	6.3	9.2	52.6
	136	135	134	133	132	131	129	128	126	125	124
	7.1	13.8	9.0	21.0	7.0	9.0	5.5	6.2	15.0	16.5	11.6
	123	122	121	120	119	117	115	111	110	109	108
	19.0	24.0	35.0	9.0	33.0	8.2	7.0	8.3	7.4	32.0	20.0

m/e	107	106	105	97	96	95	94	93	92	91	83
%	30.0	8.2	34.0	7.4	8.7	31.0	14.6	31.0	8.9	34.5	12.6
	82	81	80	79	78	77	71	69	68	67	65
	16.0	38.0	7.2	31.0	5.5	23.0	5.8	40.0	9.7	31.0	11.5
	59	57	56	55	53	51	44	43	42	41	40
	8.3	8.5	7.2	53.0	19.7	7.0	47.0	46.0	8.0	80.0	5.7
	39	32									
	24.0	49.2									

An accurate mass determination of the molecular ion m/e 222 was made: measured: 222.1985; calculated for $C_{15}H_{26}O$: 222.1984.

Component 21

Yellow viscous oil.

Refractive index: $n_D^{20} = 1.5401$

Relative retention time: 4.93

Relative retention time of trans-2,3,4,5-tetramethoxypropenylbenzene: 5.00 (52).

Ultraviolet spectrum: λ max. 260, 302 mμ.

Infrared spectrum: 2930 (s), 2830 (m), 1690 (w), 1580 (s), 1500 (s), 1460 (s), 1410 (s), 1375 (m), 1335 (s), 1230 (s), 1180 (m), 1120 (s), 1035 (m), 1000 (s), 955 (m), 830 (m), 770 (m) cm^{-1}.

Mass spectrum:

m/e	239	238	223	209	208	195	194	193	192	181	177
%	5.8	42.0	8.0	13.0	100	6.7	12.5	88.0	21.6	6.5	7.2
	165	163	150	149	148	137	135	133	121	119	118
	19.0	5.0	10.5	6.2	6.7	8.8	9.9	17.0	6.9	7.7	6.0
	115	109	107	106	105	103	95	93	91	81	79
	5.4	10.4	10.4	5.0	19.0	8.0	7.0	8.2	23.0	8.5	26.0

m/e	78	77	69	67	66	65	63	57	55	53	52
%	10.5	24.0	8.2	8.0	6.0	13.7	7.6	6.3	11.5	8.4	5.0
	51	43	41	39							
	10.3	14.0	18.0	17.0							

An accurate mass determination of the molecular ion m/e 238 was made: measured: 238.1211; calculated for $C_{13}H_{18}O_4$: 238.1205.

Component 22

Yellow viscous oil.

Infrared spectrum: 3450 (m), 2920 (s), 2840 (sh), 1720 (w), 1480 (sh), 1460 (s), 1380 (s), 1170 (m), 1135 (s), 1075 (s), 1020 (s), 992 (s), 838 (s) cm^{-1}.

Mass spectrum:

m/e	223	222	208	207	205	204	194	189	179	176	175
%	1.8	10.8	11.2	66.5	5.4	22.4	16.0	24.6	16.3	7.8	12.0
	164	163	162	161	151	149	148	147	145	139	138
	5.9	5.6	8.0	35.0	11.2	14.5	5.1	12.7	5.6	10.8	8.0
	137	136	135	134	133	131	127	126	125	124	123
	10.4	5.8	15.0	5.7	15.0	5.8	8.4	100	10.8	5.3	17.0
	122	121	120	119	117	111	110	109	108	107	106
	9.2	16.3	5.8	22.0	6.0	32.0	5.3	20.6	11.6	24.0	6.5
	105	97	96	95	94	93	92	91	83	82	81
	26.0	5.8	10.0	24.6	8.4	28.6	5.7	32.0	10.2	8.9	36.0
	80	79	77	71	69	68	67	65	59	55	53
	5.6	27.0	19.5	8.1	32.6	8.0	26.0	10.0	19.0	40.0	15.0
	51	43	42	41	39						
	48.0	54.0	5.9	72.0	22.0						

Component 23

Yellow viscous oil.

Refractive index: $n_D^{20} = 1.5078$

Infrared spectrum: 3420 (s), 2940 (s), 2825 (sh), 1638 (s), 1460 (s), 1448 (s), 1372 (s), 1255 (m), 1190 (m), 1180 (m), 1135 (m), 1065 (s), 1025 (s), 995 (s), 976 (s), 933 (s), 910 (s), 885 (s), 870 (m), 850 (w), 802 (w) cm^{-1}.

Mass spectrum:

m/e	223	222	207	205	204	191	190	189	179	176	175
%	1.72	11.5	8.5	8.9	43.0	8.5	5.2	31.0	22.0	5.0	8.5
	164	163	162	161	159	153	151	150	149	148	147
	8.0	6.0	18.4	67.0	8.5	11.5	6.3	5.9	18.0	11.5	25.0
	145	137	136	135	134	133	131	123	121	120	119
	8.4	12.6	8.5	30.0	8.0	24.0	7.5	17.0	34.0	12.6	39.0
	117	115	111	110	109	108	107	106	105	97	96
	8.2	6.7	8.0	10.3	92.0	32.0	50.0	14.0	48.0	9.0	10.0
	95	94	93	92	91	84	83	82	81	80	79
	48.0	17.0	61.0	11.5	54.0	5.5	11.5	16.0	61.0	12.6	52.0
	78	77	71	69	68	65	59	57	56	55	54
	10.0	35.0	21.0	33.0	12.6	16.0	36.0	11.5	6.7	51.0	5.5
	53	52	51	43	42	41	39	31			
	31.0	5.2	7.8	57.5	7.2	100	29.0	6.9			

An accurate mass determination of the molecular ion m/e 222 was made: measured: 222.1981; calculated for $C_{15}H_{26}O$: 222.1984.

Component 24

Yellow viscous oil.

Infrared spectrum: 3400 (m), 2900 (s), 2840 (sh), 1710 (s), 1655 (s),

1500 (m), 1450 (s), 1412 (m), 1370 (s), 1265 (s), 1235 (m), 1130 (m), 1025 (m), 886 (m), 804 (m) cm^{-1}.

Component 25

Yellow viscous oil.

Refractive index: n_D^{20} = 1.5353

Ultraviolet spectrum: λ max. 235,310,333 mμ.

Ultraviolet spectrum of aristolone: λ max. 234,310 mμ. (19).

Infrared spectrum: 2970 (s), 2940 (s), 2880 (s), 1660 (s), 1645 (sh), 1630 (m), 1605 (m), 1515 (m), 1460 (s), 1380 (m), 1360 (s), 1305 (w), 1275 (s), 1140 (m), 1120 (s), 1070 (m), 1040 (w), 1022 (w), 1008 (w), 935 (s), 895 (w), 875 (s), 840 (w), 805 (w), 750 (w) cm^{-1}.

Mass spectrum:

m/e	219	218	204	203	192	189	185	177	176	175	163
%	11.4	61.5	9.7	63.0	9.0	5.7	11.4	5.9	27.0	39.0	9.4
	162	161	159	151	150	149	148	147	145	143	138
	17.0	46.0	9.0	5.4	5.9	10.0	13.0	46.0	11.4	6.4	5.7
	136	135	134	133	132	131	129	128	123	122	121
	6.0	15.7	9.4	27.0	5.4	14.0	8.6	8.6	9.2	14.0	21.0
	120	119	118	117	116	115	111	110	109	108	107
	11.4	49.0	54.5	17.0	7.0	15.7	5.7	5.9	21.0	14.0	23.0
	106	105	104	103	96	95	94	93	92	91	89
	11.4	56.0	7.4	12.0	13.0	15.7	6.4	30.0	11.4	81.0	5.7
	83	81	80	79	78	77	69	67	66	65	63
	6.3	21.0	8.0	39.0	18.5	54.0	20.0	27.0	8.0	27.0	10.4
	57	55	53	52	51	50	43	42	41	40	39
	5.0	36.0	36.0	11.4	21.0	5.5	24.0	9.0	100	11.5	56.0

An accurate mass determination of the molecular ion m/e 218 was made: measured: 218.1677; calculated for $C_{15}H_{22}O$: 218.1671.

3.6.0.0 Infrared Spectrum of Reference Compounds

An infrared spectrum of thin films of authentic samples of the following compounds were recorded:

(a) isobornyl acetate: 2940 (s), 2860 (s), 1725 (s), 1470 (sh), 1449 (s), 1380 (sh), 1360 (s), 1245 (s), 1238 (s), 1190 (s), 1110 (s), 1053 (s), 1020 (s), 980 (s), 837 (m) cm^{-1}.

(b) isoeugenol: 3465 (s), 3400 (sh), 2980 (m), 2920 (s), 2800 (m), 1590 (s), 1500 (s), 1460 (s), 1445 (s), 1420 (s), 1360 (s), 1260 (s), 1232 (s), 1205 (s), 1150 (s), 1120 (s), 1030 (s), 963 (s), 856 (s), 822 (s), 802 (s), 788 (s), 757 (m), 734 (m) cm^{-1}.

(c) β-terpineol: 3420 (s), 3340 (s), 2945 (s), 2865 (s), 2825 (sh), 1640 (m), 1440 (s), 1370 (s), 1290 (m), 1220 (s), 1150 (s), 1130 (s), 1045 (s), 1022 (s), 950 (s), 912 (s), 885 (s), 837 (s), 800 (s), 775 (m), 755 (m) cm^{-1}.

(d) geraniol: 3340 (s), 3280 (s), 2940 (s), 2875 (s), 2700 (sh), 1654 (s), 1430 (s), 1370 (s), 1230 (m), 1175 (m), 1105 (s), 1089 (s), 1000 (s), 830 (s), 770 (w), 742 (w) cm^{-1}.

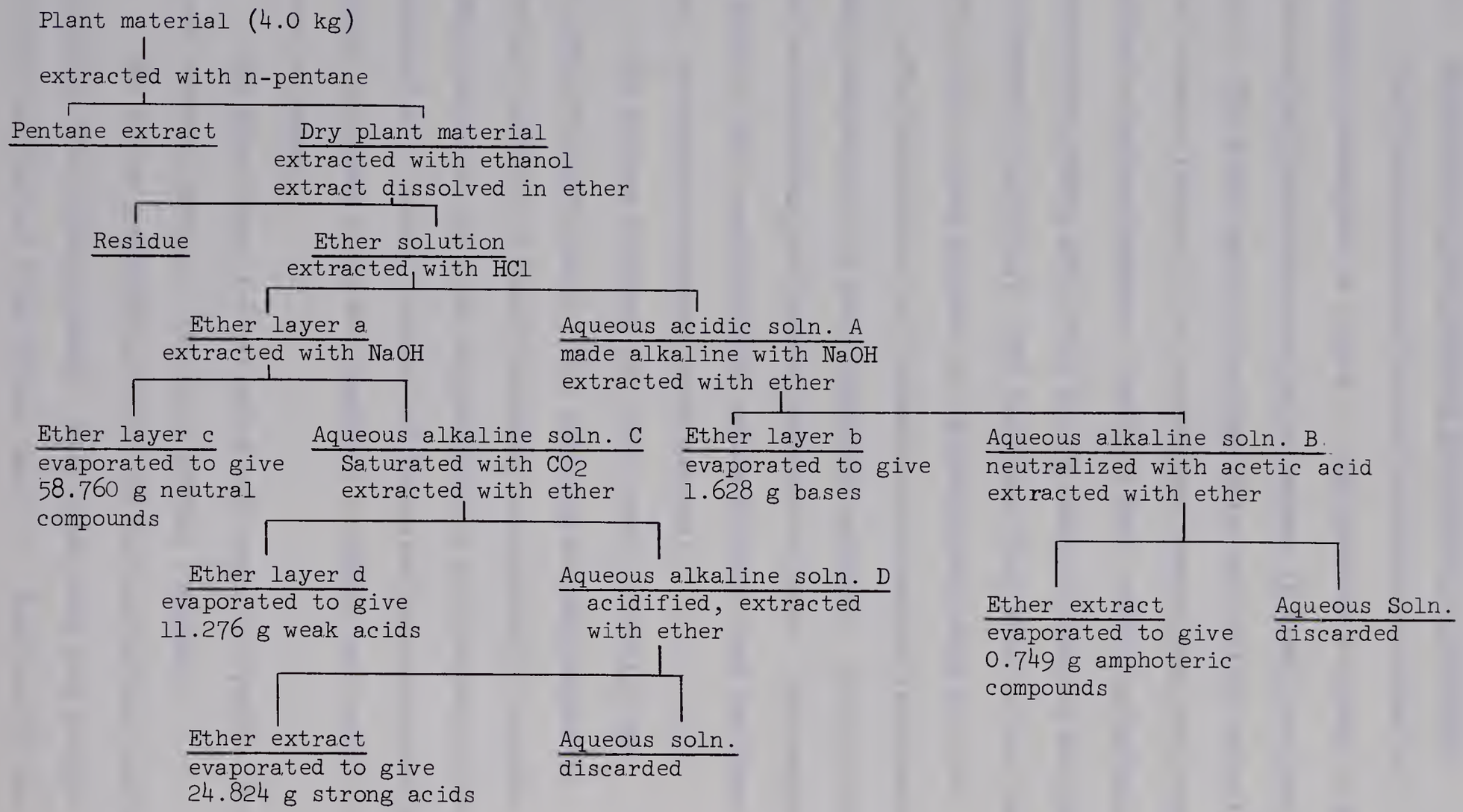
(e) isopulegol: 3395 (s), 2900 (s), 2830 (sh), 1640 (s), 1440 (s), 1370 (m), 1100 (m), 1052 (s), 1027 (s), 930 (m), 890 (s), 845 (s) cm^{-1}.

(f) limonene: 3050 (m), 2900 (s), 1640 (s), 1445 (s), 1370 (s), 1153 (s), 1050 (s), 1020 (s), 957 (s), 915 (s), 890 (s), 797 (s), 788 (sh), 758 (m) cm^{-1}.

(g) p-cymene: 2940 (s), 2840 (sh), 1505 (s), 1460 (s), 1380 (s), 1360 (s), 1215 (s), 1105 (s), 1060 (s), 1020 (s), 815 (s), 720 (s) cm^{-1}.

(h) fenchyl alcohol: 3340 (s), 2925 (s), 2850 (sh), 1450 (s), 1380 (m), 1360 (m), 1270 (s), 1210 (s), 1168 (s), 1105 (s), 1080 (s), 1065 (s), 1010 (s), 994 (s), 974 (s), 915 (s), 903 (m), 850 (s), 824 (s), 798 (s) cm^{-1}.

Figure 2. Extraction and Fractionation Scheme Used for A. Canadense Rhizomes



3.7.0.0 Extraction of the Powdered Rhizomes of A. canadense

The following extraction and fractionating scheme is schematically summarized in Figure 2. The powdered rhizomes (4.0 Kg) were exhaustively extracted with hot n-pentane (1 week) in a Soxhlet apparatus. The plant material was then dried and further extracted with hot ethanol (46 hrs). The solvent was removed from the ethanol extract under reduced pressure, leaving a dark, thick, syrupy mass. The residue was shaken with ether for 24 hours to dissolve all ether soluble material (110.28 g). The resulting ether solution was then extracted with several portions of 5% hydrochloric acid solution.

The hydrochloric acid extracts were combined (solution A) and made alkaline with 5% sodium hydroxide solution. The resulting mixture was extracted with several portions of ether. The combined ether extract, (b), was dried with sodium sulphate, and the ether removed. The residue contained basic material.

The aqueous solution (B) after ether extraction was neutralized with acetic acid. This solution was then extracted with several portions of ether to remove any amphoteric compounds.

The ether layer (a) from the hydrochloric acid extraction was now extracted with several portions of 5% sodium hydroxide solution. An emulsion was formed which was separated by adding more water and a few mls of ethanol.

The ether layer (c) was dried with sodium sulphate. The ether was removed leaving a residue of neutral compounds.

The sodium hydroxide solution (C) was cooled overnight and then saturated with carbon dioxide. Weak acids were then removed with several portions of ether (ether layer d). Solution (D) was

warmed to remove any dissolved ether, and then acidified and cooled; any strong acidic material present was removed by extraction with ether (63).

3.8.0.0 Thin Layer Chromatography of Strong Acidic and Basic Fractions from A. Canadense

The coated plates were developed by the ascending technique in a tank designed for TLC. Compounds (in chloroform) were spotted 2 cm from the bottom edge of the plates and the developing solvent was allowed to ascend a distance of 10 cm.

Two developing solvents were used; acetonitrile: diethylamine: water (8:1:1) (31) for the acid fraction, and butanol: acetic acid: water (4:1:1) for the basic fraction.

The developed plates were visualized under ultraviolet irradiation and then sprayed with bromocresol green for the acid plates, and iodoplatinate for the basic plates. Nine acidic compounds were visualized with R_f values of 0.99, 0.96, 0.82, 0.77, 0.62, 0.60, 0.42, 0.31, and 0.27. Seven basic compounds were visualized with R_f values of 0.89, 0.85, 0.80, 0.58, 0.52, 0.42, and 0.18.

IV SUMMARY

(1) The number of constituents in Canadian snake-root oil has been determined by analytical gas chromatography. Three different samples of oil were found to contain the same 30 constituents, but the quantities of each varied widely from sample to sample.

Twenty-five components of the oil were isolated by preparative gas chromatography. Ten components were positively identified, primarily by mass, and infrared spectrometry. These compounds were: myrcene, β-pinene, linalool, linalyl acetate, α-terpineol, bornyl acetate, eugenol, methyleugenol, cis-methylisoeugenol, trans-methylisoeugenol. The following five compounds were tentatively identified: β-ocimene-X, 2,3,4,5-tetramethoxyallylbenzene, cis-2,3,4,5-tetramethoxypropenylbenzene, trans-2,3,4,5-tetramethoxypropenylbenzene, aristolone. Authentic samples were not available to confirm the identity of the five compounds above.

Four compounds, which were previously reported (18,19) present in Canadian snake-root oil, were not detected in the 25 components isolated during the present investigation. These compounds are geraniol, limonene, elemicin, and 3,4-dimethoxy-cinnamaldehyde.

β-pinene, cis-methylisoeugenol, and trans-methylisoeugenol have not been reported previously for this oil.

(2) An ethanol extract from the rhizomes of A. canadense was separated into five fractions as follows: basic compounds, neutral compounds, amphoteric compounds, strong acids, weak acids. The

strong acids and basic fractions were subjected to thin layer chromatography. Nine spots were visible on the acid plate, one of which appears to be aristolochic acid, which has previously been reported present in A. canadense (29-31). The basic plate showed seven spots, one of which could be berberine; previously found in other Aristoclochia species as a contaminant.

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